STAIN TECHNOLOGY

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PROGRESS IN THE STANDARDIZATION OF BIOLOGICAL STAINS

INCREASED DEMAND FOR CERTIFIED STAINS

An unexpected increase in the demand for certified stains has occurred during the last year or more. It is believed that this is due very largely to national defense measures and other problems presented by the European war. The situation that has arisen seems to deserve more than passing comment.

It is also a matter of interest at the present time to recall that the Commission on Standardization of Biological Stains is a war baby—or, rather was once a war baby, as it was born following the first world war. The situation at that time was that previous to the war all the biological stains used in this country were imported, and the importation was practically shut off after 1914. The dealers in biological supplies were pretty well stocked up in these items, however, so the shortage was not especially felt until 1917 when the United States began to take part in the war. By this time a dye industry had been established in America, but no special effort had been made to produce the type of dyes needed in biological work. Accordingly, in 1917, when the Government began ordering stains to equip laboratories in hospitals and training camps, these orders were filled very largely with unstandardized dyes of domestic manufacture. Those who worked in any of these laboratories at that period still recall the unsatisfactory results obtained with such dyes.

It has already been mentioned in these pages[1] that the present situation is very different. Practically all the biological stains are now available in reliable quality and this quality is indicated by the Commission certification. It is very clear that the Government now realizes this situation, because practically all of the orders received by the stain companies from purchasing agencies of the Army and Navy are now for certified stains. The Stain Commission has no official status, but such orders show that it now has considerable recognition on the part of the Government.

A few figures can be cited which give a little idea as to how the war

[1]Conn, H. J. 1940. Biological stains in time of war. Stain Techn. 15, 1–2.

has stimulated the demand for certified stains. In 1934 and 1935 the number of certification labels furnished the manufacturers was approximately 20,000 each year. In 1936 it was approximately 26,000, and essentially the same number in 1937. In 1938 the figure was 29,817; these pre-war figures undoubtedly represent merely a natural increase in the demand. In 1939, however, 36,061 labels were furnished the manufacturers; and as the greatest demand followed the outbreak of hostilities in Europe, it is felt that the war had some influence in causing the increase. In 1940 the figure jumped to 67,543 and approximately two-thirds of this number were furnished the companies during the last half of the year. The figures for the first half of 1941 are not complete yet, but they are approximately the same as for the last half of 1940. It is very interesting that the most striking jump occurred after the national defense measures were begun in the United States.

This increased demand has presented its problems, altho they are distinctly different from those that were faced at the time of the previous war. The demand on the stain manufacturers is proving so great that they are having to prepare batches with unusual haste and sometimes under conditions that do not permit as high quality as usual. Thanks, however, to their coöperation with the Stain Commission, any unsatisfactory stains that result from these conditions are either kept off the market or are at least sold without certification.

A still different type of problem is faced at present in this work and has also resulted from the war. Some users of stains had, until 1940, been continuously employing German products, because they were not able to duplicate their results with stains of American manufacture. This was notably the case in regard to basic fuchsin and Giemsa stain. Now that the German stains are no longer available, such laboratory workers are faced with new problems and several of these have been brought to the attention of the Stain Commission. The majority of these problems are being rapidly solved, and the result undoubtedly is going to be an even greater standardization of the American product. It must be remarked, however, that complaints of this nature ordinarily do not indicate a fault of the American stains so much as the fact that the latter are sometimes adapted to different procedures from those previously imported.

Commission members and stain company employees have given considerable time during the last six months to an investigation of these problems. It seems better, however, that the problems be met and solved in the Commission laboratories than that they be left to face technicians at Army and Navy hospitals and training camps as was the case in 1917.—H. J. Conn.

DYE ADSORPTION BY BACTERIA AT VARYING H-ION CONCENTRATIONS[1]

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Abstract.—**Adsorption of hydrogen ions and dye cations by washed bacterial cells shows a reciprocal relationship. Apparently, H-ions and crystal violet ions are held by the cell at the same adsorption centers, and the influence of H^+ on basic dye adsorption is one of direct competition or replacement. The adsorption of H^+ and acid fuchsin is similar in that an increase is noted as the pH of the suspension is lowered.**

Hydrogen ion concentration is known to influence markedly the adsorption of dyestuffs by biological materials (Kindred, 1935; Craig and Wilson, 1937). The relative adsorbability of acid and basic stains at different H-ion concentrations depends upon the isoelectric point of the material employed (Stearn, 1933). At pH values higher than the isoelectric point basic dyes are adsorbed while at lower values acid dyes are adsorbed. This principle, used originally for demonstrating the isoelectric point of gelatin (Loeb, 1922) has been employed for approximate determinations of isoelectric points of bacteria (Tolstoouhov, 1929) as well as of other protoplasmic materials. Levine (1940) found that the staining intensity of protozoan cell structures at controlled pH-values varied not only with different dyes but also with dye or buffer concentrations, and that the isoelectric points obtained by plotting stain intensities for different pH-values also varied. He concluded that the present lack of knowledge of dye-protein combinations prevented determination of true isoelectric points by staining at controlled pH-values.

Since it has been shown that bacteria adsorb cations (McCalla, 1940) and that this phenomenon can be measured quantitatively, the staining of washed bacterial systems at different pH-values appears to offer a means of measuring the quantitative relationships involved in adsorption of dye and of H^+.

In order to visualize some of the adsorption possibilities in staining procedures, the salient physico-chemical responses of microörganisms are reviewed briefly. Normally, bacteria possess nega-

[1]Contribution No. 202, Department of Bacteriology. Coöperative investigations with the Division of Soil Microbiology, Bureau of Plant Industry.

tive charges, and therefore attract positively charged ions. Under favorable cultural conditions, the attracted bases are ions such as K^+, Ca^{++}, and Mg^{++}, which are essential to biological activity. Such ions fall near the beginning of the Hofmeister lyotrophic series and are relatively easy to replace. When bacteria possessing such ions at their adsorption sites are exposed to basic stains, dye cations possessing greater energy of adsorption can be expected to replace the metal cations. If, however, ions of high energy of adsorption, i.e., H^+, occupy the adsorption sites, the dye cations should meet with strong competition; hence, the number of dye cations adsorbed should show an inverse relationship to the H-ion concentration. When sufficient H^+ is present the charge of the bacterial system becomes neutralized and the isoelectric point is attained. As the system becomes positively charged with the addition of more H^+, it should become increasingly capable of adsorbing negatively charged ions.

It is the purpose of this investigation to show the quantitative adsorption of acid and basic dyes by bacteria as influenced by H^+ and to attempt an explanation of the phenomenon on the basis of an adsorption exchange process.

Experimental

Crystal violet[2] and acid fuchsin were made up in 0.01 *M* solutions. Aliquots of the dye solutions were adjusted to pH 0.60, 1.00, 1.50, 1.90, 2.50, 2.90, 3.98, 4.90, 6.95 and 9.10 with HCl or NaOH as needed. All pH determinations were made with the glass electrode. Bacterial cells for dye adsorption were prepared from one-day nutrient agar[3] cultures of *Corynebacterium simplex* (Amer. Type Culture Collection No. 6946) and *Bacillus bellus* (Kansas State College stock culture). Surface growth was suspended in distilled water and then washed three times by means of an angle centrifuge. After the final washing, cell suspensions were adjusted to turbidity corresponding to 25 times that of McFarland's nephelometer Tube No. 2. Portions of such cell suspensions were adjusted as nearly as possible to the pH values of the dye solutions.

Employing dye solutions and bacterial suspensions of corresponding pH-values, 1 ml. to 3 ml. of dye solution were added to 5 ml. of bacterial suspension. After thoroly mixing, the bacteria were

[2]Crystal violet 89% dye-content, and acid fuchsin 60% dye content. Of the former 4.581 g., and of the latter, 9.756 g., were used to make 1000 ml. of aqueous solutions Dyes used were certified by Commission on Standardization of Biological Stains.

[3]Agar, 25 g.; peptone, 10 g.; beef extract, 3 g.; NaCl, 5 g.; water to make 1000 ml.; adjusted to neutral reaction with NaOH.

thrown out of the suspension by means of the centrifuge, and the pH value of the remaining solution was determined.

After centrifuging, unadsorbed dye was determined by diluting 1 ml. of the clear fluid with sufficient 2% KH_2PO_4 to assure a readable color range, then comparing in the colorimeter with a standard dye solution similarly treated except for exposure to bacterial cells. Adjusting all unadsorbed dye solutions to the same pH-value by means of the phosphate solution enabled fairly accurate dye measurements. Difference between original dye solution and the unadsorbed, or recovered dye solution, was considered as the amount of dye adsorbed by the bacterial system. The amount of dye adsorbed was expressed as m. mol. (millimolar) per 100 g. bacteria dried at

TABLE 1. THE ADSORPTION OF CRYSTAL VIOLET, ACID FUCHSIN, AND H^+ AT DIFFERENT pH VALUES BY *Corynebacterium simplex* AND *Bacillus bellus* AS M. MOL. PER 100 G. BACTERIA.

Crystal violet				Acid fuchsin				H^+			
C. simplex		*B. bellus*		*C. simplex*		*B. bellus*		*C. simplex*		*B. bellus*	
pH of dye-bacteria	m. mol. adsorption	pH of dye-bacteria	m. mol. adsorption	pH of dye-bacteria	m. mol. adsorption	pH of dye-bacteria	m. mol. adsorption	pH of HCl-bacteria	m. mol. adsorption	pH of HCl-bacteria	m. mol. adsorption
0.58	19.0	0.80	0.0	0.52	15.6	0.89	21.5	0.75	119.0	1.36	77.2
1.09	30.8	1.30	3.1	1.00	20.2	1.49	30.4	1.35	116.0	2.65	75.0
1.62	51.3	1.82	14.1	1.50	21.0	1.90	33.2	3.80	64.5	4.20	48.6
1.96	68.6	2.17	24.6	2.00	17.6	2.30	33.3	6.00	22.0	5.35	25.2
2.46	80.0	2.70	20.8	2.54	11.6	2.85	30.5	6.80	16.1	6.00	20.0
2.84	86.6	3.35	34.8	3.15	10.2	4.00	14.2	—	—	6.55	15.5
3.65	95.0	4.20	63.3	4.40	7.4	4.95	7.4	—	—	—	—
3.94	99.6	5.12	98.3	4.94	5.0	5.10	5.1	—	—	—	—
5.95	109.0	6.30	106.0	6.73	4.6	7.50	1.4	—	—	—	—
8.40	109.0	7.79	109.0	—	—	—	—	—	—	—	—

105° C. Hydrogen ion adsorption was measured by the method of McCalla (1940).

The adsorption of acid and basic dyes and of H^+ by *Corynebacterium simplex* and *Bacillus bellus* is shown in Table 1. Adsorption curves for the latter are shown in figure 1. Altho the results are presented from a single experiment, many similar experiments over a six-months period reveal the data shown to be representative and subject to duplication within narrow limits.

In the pH-range suitable for basic dye adsorption, mixtures of basic dye solution and bacteria revealed pH-values of from 1 to 2 units lower than the values of either the dye or the bacterial components of the system taken separately. Representative decreases

are shown in table 2. Data showing the increased m. mol. adsorption with increased dye concentrations are given in table 3.

Discussion

It is apparent from the data presented that under H-ion concentrations characteristic of normal growing conditions, the microorganisms studied possessed a high capacity for basic dye adsorption. As the bacterial systems were made more acid, the H^+ adsorp-

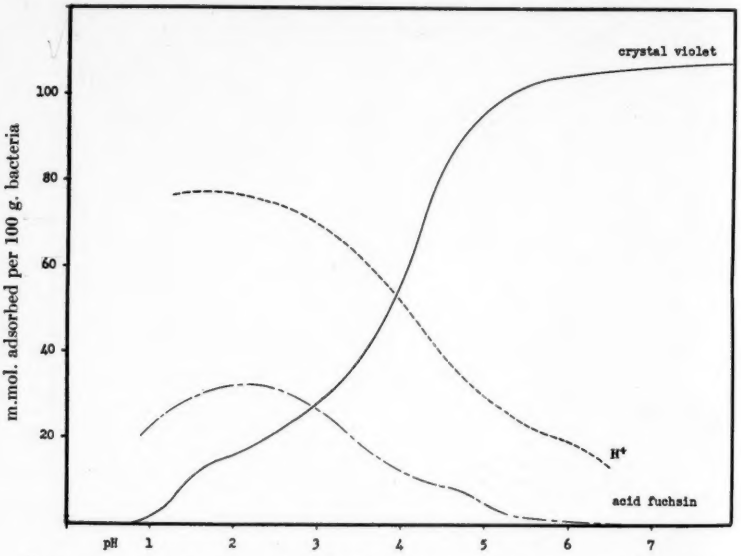

Fig. 1. Adsorption of crystal violet, H^+, and acid fuchsin by *B. bellus* at different pH values.

tion increased and the ability of the bacterial cells to take up crystal violet decreased; there was a reciprocal relationship between the adsorption of H^+ and of crystal violet. Gieseking and Jenny (1936) have shown that the energy of adsorption of H^+ and methylene blue is approximately the same.

It was observed that in the range of approximately pH 4 to 8, or in the range suitable for basic dye adsorption, mixtures of dye solution and bacteria revealed pH values of from 1 to 2 pH-units lower than the values of either the dye or the bacterial components of the system taken separately. Such sharp drops in pH values in dye-bacteria systems indicate that when crystal violet is adsorbed, it

displaces adsorbed H^+. In view of such displacement, it is believed that cations are adsorbed at the same sites, and that the adsorption of one ion influences the adsorption of another because of competition for adsorption sites. Such an assumption permits a simple explanation of the action of the factors which have been shown to influence staining intensity, as kind and concentration of dye and nature and concentration of buffer salts. The effective competition of any given dye, as revealed either by staining intensity or by milli-molar adsorp-

TABLE 2. THE DECREASE IN pH VALUE AS INFLUENCED BY CRYSTAL VIOLET ADDITIONS TO *Corynebacterium simplex*.

	pH readings		
Bacterial suspension	Dye solution	Dye-bacteria mixture	Decrease due to mixing
3.72	4.20	3.03	0.69
4.14	4.20	2.97	1.07
5.03	5.15	3.48	1.55
5.50	5.50	3.80	1.70
6.50	6.15	4.80	1.70
8.48	8.53	6.60	1.88

TABLE 3. ADSORPTION OF CRYSTAL VIOLET BY *Bacillus bellus* AT DIFFERENT DYE CONCENTRATIONS.

pH of bacteria	pH of dye	m. mol. of dye added	pH of mixture	Decrease in pH	m. mol. of dye adsorbed
7.12	6.95	45.4	6.05	0.90	45.0
7.12	6.95	90.8	6.00	0.95	84.5
7.12	6.95	136.3	5.86	1.09	110.0
7.12	6.95	181.7	5.76	1.19	115.0

tion, should become greater as its concentration increased; this has been found true in the present work. It is also logical that different dye cations should compete with H-ions with varying degrees of success, as do the ions of the Hofmeister series. Likewise, the presence of other ions, from buffer salts or other sources, should be expected to compete with dye cations with varying success according to their nature and concentration.

A relationship is seen between the adsorption of acid fuchsin and of H^+, in that adsorption for both increases at the lower pH-values. The work of Winslow and associates (1924) on electrophoresis of bacteria has shown that the addition of sufficient H^+ neutralizes the normally negative charge of bacteria. It is probable that with the adsorption of sufficient H^+, or of other strongly attracted cations, the bacterial cells acquire a positive charge, and that correspondingly,

acid fuchsin or other anions are adsorbed. The influence of H-ion concentration on acid dye adsorption would therefore be the result of changing the charge of the bacterial system, whereas its influence on basic dye adsorption is one of direct competition for adsorption sites.

Data of the nature accumulated indicate that quantitative factors can be established for dye adsorptions, and that recognition of certain fundamental factors may eventually permit regulating the concentration of H^+ and of other ions sufficiently well to obtain maximum, medium, or minimum adsorption of either acidic or basic dyes by bacteria.

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FROZEN SECTION MICRO-INCINERATION

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ABSTRACT.—**A method for micro-incineration of frozen sections is described. Material containing diffusible or soluble salts is cut on the freezing microtome and the sections are placed into xylol and mounted out of xylol onto Corex D slides previously filmed with glycerin-gelatin medium. Material containing non-diffusible or insoluble salts can be fixed in 10% formalin before sectioning. Sections of the fixed material are dehydrated thru 50, 70, and 95% ethyl alcohol and mounted out of absolute alcohol onto Corex D slides previously filmed with glycerin-gelatin medium. After mounting by either procedure the sections are incinerated in an electric furnace and the temperature of incineration is dependent on the type of tissues to be incinerated and the character of the salts present. The method is time saving and when no fixation is required the whole procedure can be carried out in one hour.**

The use of micro-incineration has found a varied application in scientific research since its introduction by Raspail. In this laboratory it has been used extensively and many instances have necessitated the development of new technic. It is our purpose to describe such new developments.

In animal skin research there are inorganic constituents which are of great interest both from a biological and a biochemical viewpoint. In such skins as are potential leather, the presence and condition of the physiological salts influence the skin's inherent quality and also its preservation during "cure". Cure is dehydration by NaCl with the mechanical draining off of some of the albumins and the globulins present.

The amount of physiological salts present is very small, amounting to less than 2% on the native weight of the hide; and as it is so universally distributed thruout the entire substance of the skin, its demonstration requires careful technic. Any appreciable losses by solvents would greatly alter the ash pattern obtained by micro-incineration. Therefore, we have developed for the demonstration of the physiological salts or other readily diffusible salts in skin or in other tissue structures, a simple technic which not only preserves the salts present but also expedites the process.

Fixation. If the salts present are not soluble in formalin and the specimen cannot be sectioned immediately, it is fixed in a 10% formalin-water solution. In the instance where diffusible or soluble salts are present, no fixation is made because the salts will diffuse

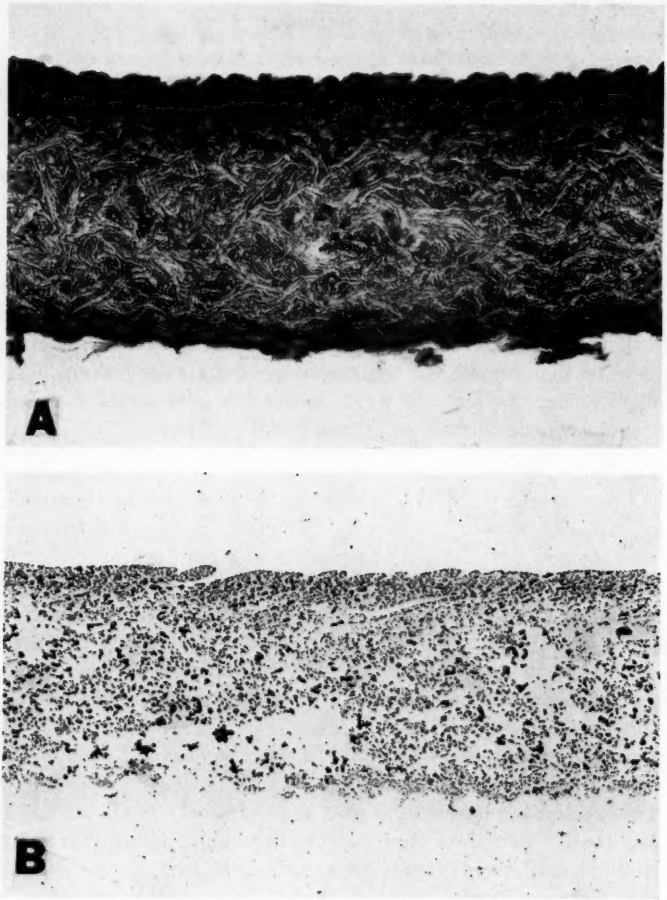

Fig. 1. A. Cross-section of chromed leather stained with hematoxylin. B. Neighboring cross-section after incineration.

out of the specimen into the fixing solution.

Sectioning. Either the fixed or native specimen is sectioned on a freezing microtome using the minimum of water to freeze it. In the case of soluble salts the cut section, approximately 20 μ thick, is im-

mediately transferred to xylol and then mounted on a Corex D slide which already has been filmed with glycerin-gelatin medium. After removing the excess medium from around the section, it is flattened out on the slide, by pressing with filter paper, and is ready for incineration. If the salts to be demonstrated are not soluble, the frozen section can be placed into water after cutting. It is then dehydrated in 50, 70, and 95% ethyl alcohol and mounted onto a glycerin-gelatin filmed Corex D slide out of absolute alcohol. Here again the section is flattened out on the slide by pressing with filter paper, and is then ready for incineration.

Incineration. The temperature to be attained for complete incineration depends upon the character of the salts present and the nature of the tissues of the section. This is only determined by experience with the type of sections being incinerated. The following information pertains to the temperatures that give the best results when calf skin or steer hide sections are incinerated.

For demonstration of NaCl and physiological salts in skin we have found that the section can be placed into an electric furnace and heated to 600° C. and then removed to cool. Going much above 600° C. causes dissociation of the salts and gives fusion mixtures instead of crystals. The incinerated section after cooling can be examined immediately. No coverslip is necessary as the ash pattern will not be disrupted unless given a severe jarring.

In our field of investigation, where $Ca(OH)_2$ is used for removing hair from the skin or hide, we have used incineration as a means of studying the rate of penetration of the lime solution into the skin. Specimens are removed from the lime liquors at given intervals, fixed in 10% formalin, cut into sections on the freezing microtome, and the sections are dehydrated thru alcohols and then mounted onto slides filmed with glycerin-gelatin medium. Because of the insolubility of $Ca(OH)_2$, the specimens can be fixed in formalin with no noticeable loss of $Ca(OH)_2$.

Leather tanned in a basic $Cr_2(SO_4)_3$ solution, fixed in 10% formalin, and then put thru the procedure mentioned for the limed specimens, gives an incinerated section that shows the chromium as Cr_2O_3 when incinerated at 650° C. For demonstration of the $Cr_2(SO_4)_3$ it is necessary to use the xylol technic and incinerate at 650° C.

Examination of Micro-incinerated Sections. The incinerated sections can be examined under bright-field and dark-field illumination. As recommended by Gage,[1] a more complete picture can be obtained

[1]Gage, S. H. 1938. Apparatus and Methods for Micro-incineration. Stain Techn., 13, 25–6.

by examining neighboring sections which have been prepared and stained in the usual manner.

To demonstrate some of the pictures obtained by the technic described above, the two photomicrographs in Fig. 1 are shown. The section marked A represents a cross-section of chrome leather stained in the usual manner with hematoxylin while the section marked B shows a neighboring section after incineration. The incinerated section represents a photomicrograph taken in bright field illumination but a similar ash pattern arrangement occurs when the section is examined under dark field illumination.

The application of the frozen section method to the micro-incineration procedure is not only adaptable to animal skin but also works well on certain plant tissues. It is a rapid method and in those cases where no fixation of the specimen is necessary the whole procedure can be carried out in one hour.

THE HISTOCHEMICAL DEMONSTRATION OF HEMOGLOBIN IN BLOOD CELLS AND TISSUE SMEARS

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The demonstration of intracellular hemoglobin in permanent preparations has long been a problem. The affinity of hemoglobin for iron hematoxylin is well known but this stain also colors yolk, chromatin, and other structures and is therefore not a reliable criterion. The presence of hemoglobin has been associated with an acidophil cytoplasm which stains a characteristic color, but a careful

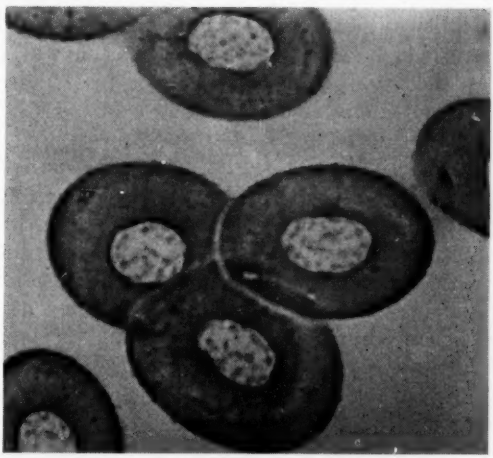

Fig. 1. Adult frog erythrocytes, stained to demonstrate presence of hemoglobin.

inspection of living cells in early hematopoetic or embryological stages demonstrates that hemoglobin is present in the erythrocytes which are quite basophilic. In the course of some research on the blood of embryonic frogs it became desirable to demonstrate the presence of hemoglobin in cells by means of a specific staining reaction.

Lison,[1] Slonimski[2] and others have described methods for the

[1]Lison, L. 1930. Une technique de détection histochimique de l'hémoglobine. Compt. Rend. Soc. Biol., **103**, 36–8.

[2]Slonimski, P. 1931. Recherches expérimentales sur la genèse de sang chez les amphibiens. Arch. Biol., **42**, 415–77.

demonstration of hemoglobin based on the fact that benzidine in the presence of hemoglobin and water may be oxidized to a brown substance. These methods are not applicable to blood smears. The solution used by Slonimski brings about immediate hemolysis, leaving only the "ghosts" or cell membranes of erythrocytes and the leucocytes with the nucleus stained a rather dark brown. No positive test for hemoglobin is possible upon smeared material with this method. The destruction of cells is due undoubtedly to the presence of acetic acid in the solution.

The procedure for the new method is as follows: An ordinary dried blood smear is flooded with a 1% solution of benzidine in absolute methyl alcohol and allowed to stand for one minute. The benzidine solution is then poured off and replaced with a 25% solution of Superoxol in 70% ethyl alcohol. This is allowed to stand for 1½ minutes, then washed in distilled water for 15 seconds, after which the smear is dried and mounted in neutral Canada balsam. By this method all structures containing hemoglobin are colored a dark brown. Nothing else is stained. The hemoglobin in the living erythrocyte of frog blood is all in the cytoplasm (See Fig. 1). The nucleus is colorless and clear, not dark as maintained by some authors (Slonimski). If it is desirable to study other cells of the blood or the nuclei of the erythrocytes, the preparation may be stained with Wright's stain subsequent to the benzidine procedure with good results. This is useful in the study of such tissue as the spleen where portions of fragmented erythrocytes are phagocytized, or in hematopoetic tissue where red blood cells are being formed. Smears of various organs may be prepared[3] by teasing a bit of the organ in cell free blood serum and smearing this on a slide or coverslip. The presence of hemoglobin in any cell is easily demonstrated by this method.

[3]Issacs, R. 1928. Alteration of tissue cells in the blood stream. Science, **68**, 547–8.

AN INEXPENSIVE LAMP FOR THE DISSECTING AND RESEARCH MICROSCOPE[1]

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The lamp described herein is inexpensive and serves a dual purpose. It is easy to construct and will provide good illumination for most work with either the dissecting or the research microscope (Fig. 1).

The source of light is a 60-watt bulb with a silvered neck ("neck reflecting"). The use of this type of bulb obviates the need for a reflector in the lamp since the light is concentrated at the end of the

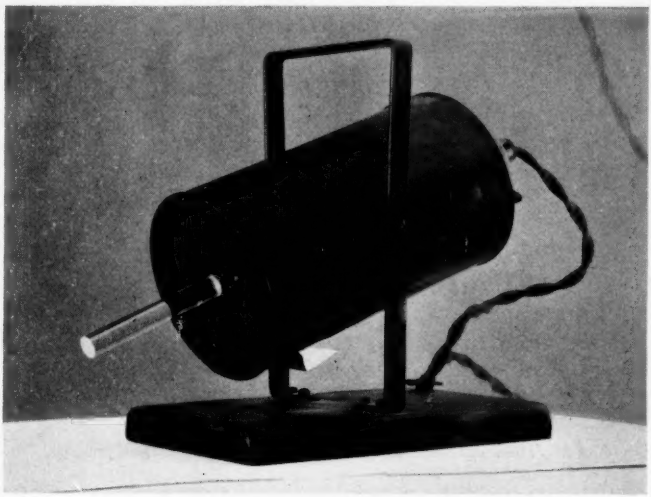


Fig. 1. The finished lamp, assembled.

bulb. A glass rod, 5 inches long by $\frac{7}{16}$ inch in diameter, is used to carry the light from the surface of the bulb to the object to be illuminated. To provide an even field of light, the end surfaces of the rod are ground flat on a carborundum wheel and finished with emery or fine carborundum powder. Probably a rod of lucite could, to ad-

[1]Contribution No. 648, Division of Botany and Plant Pathology, Science Service, Dominion Department of Agriculture, Ottawa, Canada.

vantage be substituted for the glass one, altho this has not actually been tried.

The lamp-house is made from a tin can, 4 inches in diameter by 6½ inches deep (a quart motor-oil tin will answer these specifications). The top of the tin is cut away and a hole to take the glass rod is made in the center of the bottom. A collar approximately 1¼ inches long of galvanized iron or sheet brass, made to provide a snug sliding fit around the rod, should be soldered in the hole to hold the rod in position. For ventilation, a rectangular opening of about 1 × 1¼ inches is cut lengthwise in the underside of the can near the bottom. In making this opening, the tin is cut along the sides and upper end.

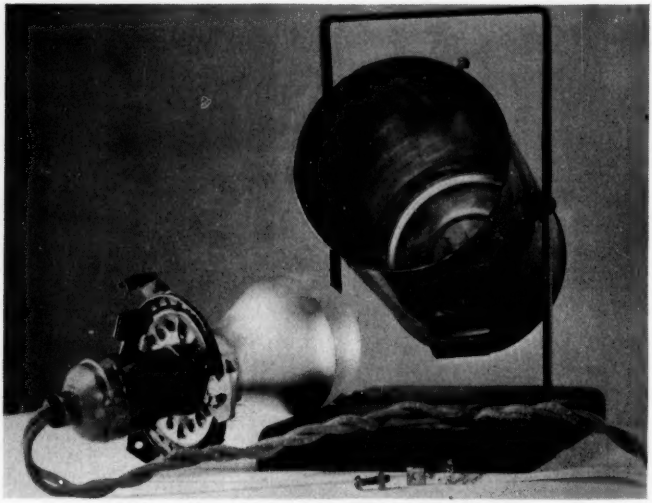


Fig. 2. The lamp with light socket and bulb removed from housing.

This leaves a flap which is bent down about ¼ inch and then upward to provide a shield over the opening. The light-socket holder is made from a smaller tin can (one about 3 inches in diameter will serve). The sides are cut away, three strips each about one inch long being left attached at equal distances around the periphery to serve as brackets. A hole, large enough to allow the end of the socket to be screwed thru it, is made at the center of the holder, and small holes for ventilation are punched around this. The socket and bulb are assembled in the holder and this unit is then bolted to the open end of the can with small stove bolts (Fig. 2). An alternative method of constructing this holder would be to screw a shade holder

to the socket and then fit this to the open end with a flange, provision of course being made for ventilation.

The lamp support, which also serves as carrying handle, is made from a strip of strap iron $\frac{1}{8} \times \frac{1}{2}$ inch, which is screwed to a wooden base 5×7 inches in size. The lamp-house is bolted to the support with stove bolts, with a small spring or spring washer on the inside to provide sufficient friction to hold the lamp-house in any desired position.

When using the light with the dissecting microscope, the end of the glass rod is brought as close to the object to be examined as is feasible without its entering the field of view; with the research microscope, the end of the rod may be brought under the stage and rested on the mirror or it may be set back at any convenient distance from it. Some adjustment of light intensity may be obtained by moving the rod in toward, or out from the surface of the bulb. For critical work, light filters and diaphragms may be affixed to the end of the rod.

CLEARING TISSUE WITH MIXTURES OF TRIBUTYL AND TRI-O-CRESYL PHOSPHATES

RICHARD A. GROAT, *Department of Anatomy, University of Wisconsin, Madison, Wis.*

ABSTRACT.—**Mixtures of tributyl phosphate (Commercial Solvents Corp.) and tri-o-cresyl phosphate (Eastman Kodak Co.), of varying proportions, may be used as immersion fluids for clearing gross specimens. By varying the ratio of the former to that of the latter (measured volumetrically) from 1:130 to 22:110, mixtures having refractive indices varying from 1.555 to 1.534 can be made. A table of mixtures and the index of each is given in this paper. Preparation of specimens consists of skinning, eviscerating, fixing, washing, bleaching (when necessary), dehydrating, and defatting. The proper clearing mixture is determined by the immersion method, which entails microscopic observation of a 50 μ section of tissue to be cleared.**

INTRODUCTION

A method is proposed for clearing gross anatomical specimens by immersion in a mixture of two chemical compounds, the refractive index of which can be made the same as that of the tissue which it is desired to clear. The method does not alter the fixed, dehydrated specimen. The cleared material is firm, can be dissected, and can be prepared for histological study. The liquids used are not toxic unless taken internally. Contacts with the skin, such as occasioned in following the method, are not harmful, but the material should be washed off when the work with it is finished.

All methods of this type must be based upon the same principle, and the results achieved can be accounted for by a consideration of the optics involved in the application of that principle. Spalteholz's method[1] is of this kind. His monograph is prolix and interspersed with irrelevant material, and the theoretical treatment is inadequate. Hence a brief discussion of the optics is given here.

OPTICS

In order that an object may be seen clearly and without undue distortion, each interface between media thru which light rays travel

[1]Spalteholz, Werner. 1914. Über das Durchsichtigmachen von menschlichen und tierischen Präparaten und seine theoretischen Bedingungen, (Zweite Auflage). Leipzig.

from object to eye must be plane (or spherical with relatively great curvature) and each interface must be of such extent that all light rays from object to eye strike it. Or, if an interface does not meet these requirements, the two adjoining media must have the same refractive index and dispersion. In addition, the light rays must escape absorption by the media and reflection at the interfaces between media of different refractive indices.

Suppose it is desired to clear an animal for demonstration of the skeleton. The animal is killed, skinned, eviscerated, fixed and placed in water. The object (bone) is surrounded by denaturated proteins, fats and water. These are media thru which light must pass to and from the bone. The interfaces, irregular and minute, are countless in number, and the three media have quite different refractive indices. Even if the bone could be illuminated, it could not be seen because light rays coming from it would undergo deviation and reflection at the myriad interfaces between the media of different refractive indices.

In order to convert such a system into one in which the bone becomes clearly visible, the following steps are taken: bleaching of pigments (if necessary), dehydrating, defatting and replacing the final solvent with a fluid whose refractive index is the same as that of the principal tissue which surrounds the bone, that is, striated muscle. Light rays leaving the bone are confronted by innumerable, tiny interfaces as in the previous case, but now the media have the same refractive indices. (Practically, it is not necessary to consider dispersion.) Hence the light rays undergo neither deviation nor reflection[2] until they meet the interface between the liquid and air. Although the liquid and air have very different refractive indices, the visualization of the object is not spoiled because the interface is plane. The bone itself is not transparent because its inorganic material has a refractive index different from that of the liquid even tho its organic material has nearly the same index. Cartilage and nervous tissue are also visible in a specimen cleared as outlined above.

Technical Procedure

Preliminary treatment of specimens. If a whole animal is used to demonstrate bone and cartilage, it should be bled thoroly, skinned and eviscerated. The refractive indices of skin and muscle are

[2]That the deviation is zero when the refractive indices are equal follows directly from Snell's law. It can be seen that the reflection coefficient, R, which for normal incidence is $\left(\frac{n_2 - n_1}{n_2 + n_1}\right)^2$ becomes zero when the refractive indices are equal.

different, hence it is impossible to clear both with the same liquid, altho both may be partially cleared by employing a liquid of intermediate refractive index. Evisceration eliminates a large amount of blood and pigment, and also food in the gut.

Fixation. A solution of commercial formalin (10 cc. in 90 cc. water) or a mixture of 5 cc. formalin in 65 cc. 95% ethyl alcohol, and 30 cc. water, is recommended. Other fixatives can be used. The fixative will decalcify if acid.

Washing. After fixation the tissue should be washed sufficiently to remove all the fixative.

Bleaching. Hydrogen peroxide, U. S. P. (3% H_2O_2), is used without dilution. If decalcification is to be prevented, the solution should be neutralized before use. An adult rat will require a week or more and probably one change of solution, while small bits of tissue should bleach in a day. The bubbles formed in the tissue can be removed by placing the specimen in water in a vacuum jar and evacuating. Any bubbles which remain after clearing can be removed by again evacuating (since the clearing fluid is not volatile) or by drawing the bubbles out with a syringe and hypodermic needle.

Washing. The tissue should be washed sufficiently to remove all H_2O_2.

Dehydration. Either acetone or dioxan can be used. A half-and-half mixture of water and dehydrating agent is followed by two changes of pure dehydrating agent, or more gradual transitions from water to dehydrating agent may be made. The dehydrating agent also removes fat. This is very necessary inasmuch as fats have a lower refractive index than proteins. Complete removal of water and of fat from an adult rat requires three or four days in each change of the pure acetone or dioxan.

Determination of the refractive index of the particular tissue to be cleared. The refractive index of a bit of tissue can be determined to the third decimal place by the immersion method. This is a method commonly employed by mineralogists.[3] The significance of this degree of accuracy in connection with the clearing of tissues is questionable because closely adjoining parts of a given tissue may vary slightly in refractive index, the refractive index of the liquid medium used changes with the temperature, and the dispersions of the liquid and of the tissues are not the same. The best medium is the one which has the same refractive index as most of the particular tissue to be cleared, at the temperature at which the cleared specimen is most likely to be observed.

[3]See Winchell, Alexander N. 1937. Elements of Optical Mineralogy, *5th Ed.* (Part I.) John Wiley and Sons, Inc., New York.

A series of liquids of different refractive indices is prepared by mixing tri-o-cresyl phosphate and tributyl phosphate (Table 1). This series covers the range of refractive indices likely to be met in tissues.

After the specimen has been completely dehydrated and defatted, a small portion of the tissue to be cleared is cut from the specimen and imbedded in paraffin by the usual procedure. The tissue may be put directly into melted paraffin from either dioxan or acetone. Sections 50 μ thick are cut. The sections at this thickness curl, and it is necessary to float them on warm water to flatten them. They are picked up from the surface of the water on clean microscope slides. No adhesive should be used. Only one slide carrying one section is required, but two slides facilitate the determination.

When the sections are completely dry, the slides are immersed in xylene to remove the paraffin. When the slide is removed from the xylene, the section is washed with several drops of xylene from a medicine dropper, and the excess xylene drained from the slide. Before the section dries, one drop of one of the refractive index liquids is placed on the section. The slide is tilted in different directions so that the liquid washes over the entire section. The drop is drained off with the aid of a blotter or paper towel, and the procedure repeated. The third drop is allowed to remain on the section. The slide is placed under the 16 mm. objective of a microscope. Normal illumination is used. The condenser should be lowered somewhat, and the diaphragm closed so that the field is rather dark. The section is brought into sharp focus. The objective is then raised very slightly with the fine adjustment. A narrow, bright line called the Becke line will be seen along every edge between tissue and liquid. If the liquid has a higher refractive index than the tissue, the Becke line will appear to move from the edge out into the liquid when the objective is raised. If the liquid has a lower refractive index than the tissue, the Becke line will appear to move into the tissue. The Becke line will be strong if the difference between the refractive indices of tissue and liquid is great, and will be scarcely noticeable if the difference is slight. If the first liquid tried is high, one having a lower index is next used. The intensity of the Becke line in the first instance will serve to indicate how much lower the second liquid should be. The first liquid is washed off with xylene from a medicine dropper, and the second liquid is applied just as the first one was.

In some cases it might be quite difficult to observe the Becke line when the refractive index of the liquid is very close to that of the tissue. This is especially true if monochromatic light is not used. In many instances the same kind of tissue in one section may vary

enough in refractive index so that the refractive index of a certain liquid may be at once higher than, lower than, or the same as that of the tissue in neighboring parts of the section as determined by the Becke line. At such times, the tissue should be assigned the refractive index of the liquid in which it displays the greatest average transparency. At the start of the procedure, while the section is still immersed in xylene, it should be carefully studied as to gross extent and as to distribution of different types of tissue. This step is indispensable. For example, a section consisting principally of striated muscle is being examined to determine the index of that tissue. When a liquid is used which has a refractive index quite close to that of the muscle, it is not possible to recognize that tissue. If the Becke line is observed, it is imperative to know whether the line comes from muscle or from adjacent collagenous connective tissue. Much of the muscle tissue may be rendered entirely invisible, and hence the extent of the section must be known in order to estimate its average transparency.

When the refractive index of the tissue has been determined thus, the entire specimen is immersed in a mixture similar to the one in the refractive index series from which the tissue index has been assigned. The specimen is transferred to the clearing mixture directly from the last change of dioxan or acetone. The container should be covered only with cheese-cloth to exclude dust until the volatile solvent has evaporated. Evacuating the preparation will hasten the removal of the solvent. If the pressure becomes too low, the tributyl phosphate may evaporate slowly but this situation can be readily recognized because the tributyl phosphate condenses on the inside of the vacuum jar.

Preparation of the refractive index series of liquids. The immersion liquids are relatively inexpensive and are prepared by mixing tri-o-cresyl phosphate and tributyl phosphate in various proportions. The tri-o-cresyl phosphate used is the technical grade sold by Eastman Kodak Company, Rochester, New York. It has a boiling point of 265° C. at 20 mm. of mercury, and a density of 1.165 g. per cc. at room temperatures. It has a very faint yellow tint. Tributyl phosphate is sold by Commercial Solvents Corporation, Terre Haute, Indiana. It boils at 178° C. at 27 mm. of mercury. Its density at room temperatures is 0.970 g. per cc. The compound is colorless. The refractive index of tri-o-cresyl phosphate is n_D^{22} equals 1.5560; of tributyl phosphate, n_D^{22} equals 1.4229. The boiling point of each of these liquids is exceedingly high. The vapor pressure of each at room temperature is practically zero. Hence, no alteration of the composition of the mixtures occurs thru evaporation at room tem-

perature. They will not vary enough among lots to affect the refractive indices of the mixtures in the third decimal place. The change of refractive index with temperature is about the same for all the mixtures. For example, n_D of liquid 17 is, at 20° C., 1.5397; at 22° C., 1.5389; at 25° C., 1.5377.

TABLE 1. MIXTURES OF TRI-O-CRESYL PHOSPHATE AND TRIBUTYL PHOSPHATE FOR USE AS IMMERSION LIQUIDS.

Number	c c. Tributyl Phosphate	c c. Tri-o-cresyl Phosphate	n_D^{22} desired	n_D^{22} determined on Abbé refractometer
1	1	130	1.5550	1.5550
2	2	130	1.5540	1.5540
3	3	130	1.5530	1.5530
4	4	120	1.5520	1.5519
5	5	120	1.5510	1.5509
6	6	120	1.5500	1.5500
7	7	120	1.5490	1.5490
8	8	120	1.5480	1.5480
9	9	120	1.5470	1.5471
10	10	115	1.5460	1.5459
11	11	115	1.5450	1.5449
12	12	115	1.5440	1.5440
13	13	115	1.5430	1.5430
14	14	115	1.5420	1.5420
15	15	110	1.5410	1.5408
16	16	110	1.5400	1.5399
17	17	110	1.5390	1.5389
18	18	110	1.5380	1.5379
19	19	110	1.5370	1.5371
20	20	110	1.5360	1.5361
21	21	110	1.5350	1.5352
22	22	110	1.5340	1.5343

In the accompanying table, 22 mixtures are listed ranging in refractive index from 1.5550 to 1.5343. Of this number, all may not be of use, but all are included for the sake of completeness. Only liquids 12 to 22 need be prepared unless others prove to be necessary. Refractive indices were determined with an Abbé refractometer. The mixtures were prepared so that each differs from its neighbors in the series by one in the third decimal place. The indices correct to the fourth decimal place, as determined with the Abbé, are also given in the table. The refractive index liquids used for testing tissues are conveniently prepared in quantities one-tenth those given in the table. Glass syringes serve well for measuring the two liquids in making up the series. The refractive index liquids should be kept in clean glass containers, and care should be taken to prevent contamination.

SUPPLEMENTARY NOTES

If accurate determinations of the refractive indices of tissues are desired, monochromatic light (the same as that for which the indices

of the liquids were determined) should be used. If, however, it is desired simply to find the liquid in which the tissue is most transparent, white light is satisfactory. It is not always necessary to make index determinations once they are learned for a given type of tissue; for example, the deep back musculature of young or adult rats has an index of 1.537 ± 0.001.

Rats, ground squirrels, bats, moles, fetal rabbits, and human fetuses have been cleared, and liquid 19 (Table 1) has served as a good clearing medium for all, altho it was not the best for some specimens.

Museum jars containing the clearing liquid can be sealed with glue, but may be merely covered because the liquid is not volatile.

The advice of Dr. R. C. Emmons, Dr. J. E. Mack and especially Dr. H. W. Mossman has been of great aid in the execution of this work.

STAINING THE NUCLEOLUS

C. S. Semmens and P. N. Bhaduri, *Kings College, University of London, Bristol, England*

About two years ago, the authors[1] described a cytological stain combining leuco-fuchsin and light green. The original technic has caused some difficulty, because the use of aqueous Na_2CO_3 in mordanting causes stripping of serial sections from the slides, unless properly hardened. While recent developments of the technic have been mainly concerned with the production of efficient and rapid smear methods (Bhaduri[2]) the use of an alcoholic mordant now does away with the need for any special treatment to avoid the stripping difficulty with serial sections.

The schedule given below can be recommended with confidence to those who wish to try the nucleolar staining method on microtome sections of either plant or animal material mounted on slides in the usual way. As previously mentioned, chrom-formalin fixatives give very good results with most plant materials used for mitosis; meiotic material may be fixed in Semmens' sodium diuranate mixture (Bhaduri and Semmens, 1940).[3] Most fixatives will, however, give good results if before hydrolysis the material is left in 1% chromic acid solution for a few hours; it should then be thoroly washed in water to remove all traces of chromic acid and left for about four hours or overnight in 75% alcohol.

Staining Schedule

1. Slides with wax ribbons are run down to water in the usual way except that it is best to leave them in the 75% alcohol grade for at least two hours.

2. Wash with warm water and leave standing in warm water for about five minutes, transfer to 12% HCl at 60° C. and keep at this temperature to hydrolyze for the optimum period, (20 seconds is a good average time to try for new or unknown plant material).

3. Remove the staining jar from the oven or heating apparatus used and immerse it in a vessel of cold water in which cooling of the slides may take place. When cold pour off the used acid and rinse

[1]Semmens, C. S., and Bhaduri, P. N. 1939. A new method for differential staining of the nucleolus. Stain Techn., **14**, 1–5.

[2]Bhaduri, P. N. 1938. Root tip smear technique and the differential staining of the nucleolus. J. Roy. Micr. Soc., **58**, 120–4. 1940. Improved smear methods for rapid double staining. J. Roy. Micr. Soc., **60**, 1–7.

[3]Bhaduri, P. N., and Semmens, C. S. 1940. Advantages of uranium fixation in modern cytological technique. Nature, **146**, 100.

the slides with fresh cold *N* HCl, pour this off and cover them with decolorized fuchsin. Leave in this for 3–4 hours or overnight, as convenient.

4. Pour off the dye and cover the slides with bleaching solution, using two changes of this with half an hour in each.

5. Rinse in distilled water, in 50% alcohol, then 70% alcohol.

6. Place slides in mordant solution, consisting of 80% alcohol saturated with Na_2CO_3; leave in this mordant for at least one hour.

7. Pour off the mordant solution and rinse the slides with 80% alcohol to remove all traces of mordant; rinse quickly in 95% alcohol and then place 20–25 minutes in a light green solution prepared as follows: after filtering a saturated alcoholic solution of light green S. F. Y. (C. I. 670), add 2–3 drops of pure colorless anilin oil per 100 cc. of dye.

8. Drain off the dye and rinse the slide, for preliminary treatment, in a differentiating solution consisting of: Na_2CO_3, saturated solution in 80% alcohol, 10 cc.; 80% alcohol, 90 cc. Continue the differentiation in 95% alcohol, examining under the microscope to watch progress. If necessary, return to the differentiating solution for further treatment and then back to the 95% alcohol. In this way differentiation can be carefully controlled until the green is cleared from everything but the nucleoli. The last traces of green may of course be removed from the cytoplasm in the process of dehydration.

9. Dehydrate in two or three changes of absolute alcohol.

10. Clear in alcohol-xylol (50–50), xylol-alcohol (75–25) followed by pure xylol.

11. Mount in "Sira" mountant or some similar neutral medium.

As frequently pointed out, it is necessary to determine by experiment the best period and temperature for hydrolysis with any new material and a clear-cut Feulgen reaction is only to be expected if this is done. Hydrolysis of material on slides is best carried out in a porcelain Coplin jar; the thick glass of the ordinary staining jars will not stand the necessary heating and cooling treatment.

Material that has been fixed in osmium fixatives can usually be stained by the following procedure: Bleach as usual in 3 parts of 80% alcohol to 1 part of 20 vol. hydrogen peroxide. Transfer to 70% alcohol for 1–3 hours to harden, then run down to water. Mordant in 1% chromic acid as mentioned earlier, then carry on with the above schedule.

Instructions for the preparation of decolorized fuchsin and the bleaching solution may be found in previous numbers of Stain Technology.

CHLORAZOL BLACK E, A SIMPLE CONNECTIVE TISSUE STAIN

NORMAN D. LEVINE[1] and C. C. MORRILL,[1] *Animal Pathology Laboratory, University of Illinois, Urbana, Ill.*

Cannon,[2] Darrow[3] and Nebel[4] have reported that chlorazol black E is of value as a biological stain. The dye, according to Darrow, is

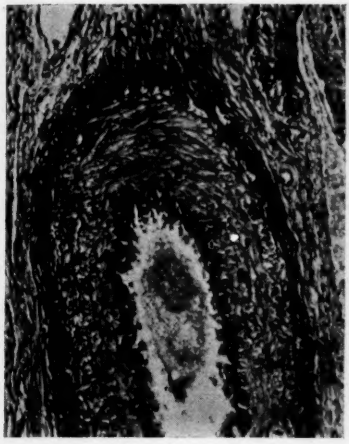


Fig. 1

Fig. 2

Slightly diagonal section of an artery (125×) stained with chlorazol black E (Fig. 1) and with hematoxylin and eosin (Fig. 2). Note the contrast in appearance of elastic fibers.

synonymous with Erie black GXOO (National Aniline and Chemical Co.), pontamine black E (I. E. du Pont de Nemours Corp.) and chlorazol black E (British Dyestuffs Corp).

Darrow recommended staining in a 1% solution of the dye for 5 to

[1]Assigned by the State Department of Agriculture to the Animal Pathology and Hygiene Laboratory to assist in diagnosis and research.

[2]Cannon, H. J. A new biological stain for general purposes. Nature, **139**, 549. 1937.

[3]Darrow, M. A. A simple staining method for histology and cytology. Stain Techn., **15**, 67. 1940.

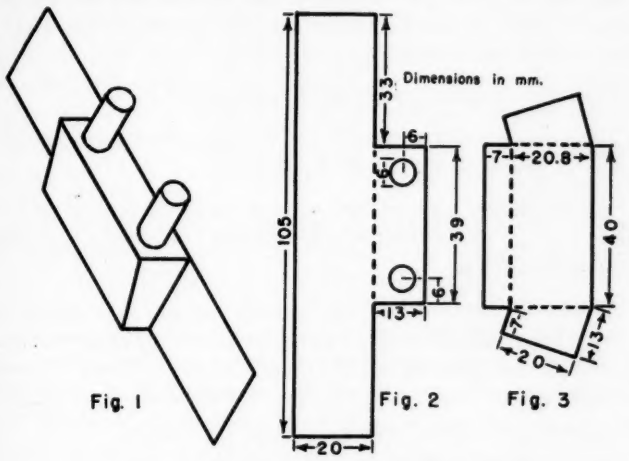
[4]Nebel, B. R. Chlorazol black E as an aceto-carmine auxiliary stain. Stain Techn., **15**, 69. 1940.

10 minutes, and then dehydrating, clearing and mounting, while Cannon suggested a saturated solution in 70% alcohol. Since a 0.5% solution in 70% alcohol of the sample used by the writers was saturated, there appeared to be no point in using a 1% solution. A staining period of 30 minutes was considered preferable to the shorter time. Neither mordanting nor destaining is necessary.

The writers have confirmed Darrow's findings that chlorazol black E gives sharp differentiation in histological and cytological preparations, and stains different tissue elements black, green or yellowish green. It is noteworthy that the elastic fibers of connective tissue stain an intense black (Fig. 1, 2). Since many of the staining procedures used in the study of these elements are relatively complicated and time-consuming, it is felt that the simplicity of the staining technic with chlorazol black E should recommend it to histologists for such studies.

NOTES ON TECHNIC

TEMPERATURE CONTROL FOR MICROTOME KNIVES.—Temperature control during paraffin sectioning is readily obtained if a safety razor blade holder with provision for water circulation is used. With a microtome knife, however, the problem is more complicated. A device described by Cooper and MacKnight[1] provides for chilling but does not seem quite as well suited for warming the knife—a procedure which is often necessary when thick sections are cut. The device shown in Figure 1 may be used to chill or warm a microtome knife by means of water circulation. The essential features are that it presents a large flat surface for close contact and heat exchange with the knife, and is very readily attached.



Figs. 1–3. Device for chilling microtome knife.

The water-jacket consists of two pieces of $\frac{1}{32}$ inch sheet copper soldered together and two nipples of $\frac{3}{8}$ or $\frac{1}{4}$ inch copper or brass tubing for the attachment of rubber hoses. The design of the component pieces is shown in Figs. 2 and 3. Dimensions (which are indicated in millimeters) may have to be altered to fit various sizes

[1]Cooper, K. W., and MacKnight, R. H. Cooling device for the microtome. Stain Techn., 12, 25–7. 1937.

and types of knives and knife-holders. If a hollow ground knife is used, the contact surface of the water-jacket must be curved to fit.

To attach the water-jacket to the knife, the ends of the contact plate are interposed between the clamp screws and the knife. Sufficient space should be left between the knife edge and the top of the water-jacket to accommodate at least one section; otherwise it may be difficult to start a ribbon.—JOHN W. DUFFIELD. Northeastern Forest Experiment Station, New Haven, Conn.

A USEFUL EMBEDDING TECHNIC—The following simple but successful method for embedding insect or other tissue in paraffin for microscopical serial section has been developed. It eliminates most of the difficulties that are experienced in embedding. It differs from the usual methods in that very hot paraffin is employed and ice cubes are used to cool the paraffin in the embedding boats. The steps in the process are as follows:

1. Kill, fix, dehydrate, and infiltrate as usual.

2. Heat the embedding paraffin and the ends of a pair of forceps in dishes over boiling water to 180° F. or above.

3. Fill an embedding boat with the hot paraffin and place it on an ice cube to cool. Two or more boats may be filled at the same time, as experience allows.

4. When the lower portion of the paraffin is completely opaque, lift the tissue by means of the hot forceps from the infiltration chamber to a dish of hot paraffin for an instant, or until the cool paraffin around the tissue is melted, and then transfer it to the embedding boat.

5. Orient and allow to cool.

The success of the method lies in the fact that the paraffin is cooled much more rapidly from the bottom than it is from the top. This allows the placing of the tissue well up in the block, exactly at the freezing point of the paraffin, without the danger of layering. The hot paraffin above and the hot forceps allow leisurely orientation of the tissue without the danger of introducing air bubbles. The rapid cooling aids in producing small paraffin crystals. No trouble has been experienced with cupping or treeing. The use of thin cotton gloves may aid some people in handling the heated forceps.—C. E. WOODWORTH, U. S. Dept. Agriculture, Walla Walla, Wash.

A METHOD FOR NUMBERING SERIAL CELLOIDIN SECTIONS.—A method for marking celloidin sections has been developed to permit staining large numbers of serial sections at one time without first mounting them on slides. This involves a marking substance which, unlike India ink and similar substances, is permanent except in solvents of celloidin.

Sections are cut and placed chronologically between slips of paper in the usual manner. An "ink" is prepared by thoroly mixing equal parts of 20% nitrocellulose (Hercules Powder Co., RS ½ sec.) in amyl acetate[1] and black oil color (e. g. Martini Studio Oil Color, Ivory Black). One corner of each section is blotted and then numbered by means of a small camel's hair brush. Before returning the sections to the alcohol, the ink is "fixed" with a drop of chloroform applied with a second brush. Numbers applied in this way become an integral part of the celloidin around the section and do not wash off. The substance here described is more viscous than those usually used, but with a little practice it can be applied as easily and rapidly as any other numbering material.—JOHN MEACHAM HAMILTON, Laboratory of Neurophysiology, Yale University School of Medicine, New Haven, Connecticut.

A FORMALIN-PHENOL-THIONIN STAIN FOR NERVOUS TISSUE.—Fresh nervous tissue or nervous tissue treated according to the Marchi technic (Swank modification) stains rapidly (1–5 minutes) and well with the usual aqueous phenol-thionin procedure.

If, however, it is fixed by perfusion with 10% formalin, and subsequently stored in this reagent, it stains very lightly in 1% aqueous phenol-thionin, even tho applied for several hours or overnight. To hasten and intensify the staining, several modifications have been tried and the following procedure adopted as most desirable.

1. Wash nitrocellulose sections in distilled water.

2. Stain 15–30 minutes at 50° C. in the following mixture: 25 cc. 0.5% aqueous thionin (National Aniline Co., C. I. 920) plus 2 drops of phenol (liquefied) and 2 drops of formaldehyde (34–38% U. S. P.).

3. Rinse in distilled water.

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[1]Nitrocellulose is sold in a dehydrated form consisting of about 10 parts nitrocellulose to 7 parts absolute alcohol. The 20% solution mentioned above is made by adding 20 g. of nitrocellulose-alcohol mixture to 80 cc. amyl acetate.

4. Wash well in 70% ethyl alcohol.

5. Place in 95% alcohol 2–4 minutes.

6. Absolute alcohol plus 12–15% of U. S. P. chloroform.

7. Clear and differentiate sections individually 1–10 minutes in equal parts of: oil of origanum, cretic; oil of bergamot, f. b. extra fine; phenol. Avoid creosote!

8. Place on clean glass slide with forceps. Low volatility of clearing fluid allows adequate time to orient serial sections. If sections become too dry or stick to the slide in improper position, dampen with a brush dipped in the clearing fluid.

9. Roll sections flat with a camel hair brush. Blot dry with bibulous paper. Complete drying and flattening process with a silk cloth wrapped around index finger.

10. Wipe around sections with a cloth moistened in xylol to remove oil droplets. Care must be exercised that the sections do not become so dry that they show silvery patches when viewed from the reverse side of the slide. Slight dampening with clearing fluid will correct this.

11. Remove any lint or dirt with a clean, dry, camel hair brush.

12. Mount in damar, clarite or balsam.

Sections are more easily stained if not stored in alcohol for more than a week. U. S. P. formaldehyde was found to give less background staining than that of reagent quality. Altho staining can be accomplished without phenol, it probably retards fading of the sections. Several hours (e.g., overnight) in 70% alcohol does no harm if sections are deeply stained. If desired, sections may be left overnight in absolute alcohol plus chloroform. The origanum-bergamot-phenol clearing fluid may be modified by adding a small amount of methyl salicylate or amyl acetate to make sections more pliable. Oil of lemon, Californian U. S. P. (XI), may be used as a differentiating fluid prior to clearing. Synthetic oil of bergamot may be used instead of the more expensive f.b. extra fine grade. Coverslip weights should be used if thick sections are mounted. Large infant brain sections, stained in formalin-phenol-thionin or by Weil's technic for myelin, are also easily mounted on large slides or between lantern slide cover slips in this manner. Nitrocellulose used in developing the above technic was procured from Charles Cooper, Manufacturing Chemists, New York. GEORGE C. GRANT, Division of Anatomy, University of Tennessee College of Medicine, Memphis.

STAIN FADING IN VARIOUS HISTOLOGIC MOUNTING MEDIA.—Sets of five sections each of monkey pancreas, adrenal, spleen, kidney, and heart were stained in Weigert's iron chloride hematoxylin and Van Gieson's picrofuchsin, using 0.1 g. acid fuchsin per 100 cc. saturated aqueous picric acid and another similar series with our buffered Romanowsky stain at pH 4.2.

One set of sections with each of the two stains was mounted in neutral Canada balsam in xylol, one set in Curtis' salicylic acid balsam, one set in 60% clarite in xylol, one set in 60% clarite X in xylol and the last set in heavy liquid petrolatum U. S. P. ($N\Delta = 1.483$) and sealed with pyroxylin cement.

The pancreas and adrenal sections were exposed continuously under a 100 watt electric light at a distance of 22–25 cm. in a box painted inside with aluminum paint and on an aluminum tray. The others were left on a north window sill in diffuse daylight for 6 months.

The blue component of the Romanowsky stain showed appreciable fading in salicylic balsam in 90 minutes and was entirely gone in 24 hours under the lamp. In 5 days, fading was moderate in neutral balsam, and in 10 days quite marked. At 10 days both clarite and clarite X showed slight fading. After 3 months under the lamp and after 6 months in daylight, the salicylic and the neutral balsam showed complete fading of the blue component, clarite X quite marked fading, clarite moderate fading, and the liquid petrolatum practically no change.

The iron-hematoxylin picrofuchsin stains, after 6 months in daylight or 3 months under the electric light, showed red collagen with clarite and clarite X, pink collagen with salicylic acid balsam and liquid petrolatum, and completely unstained collagen with neutral balsam. The nuclear staining showed a slight loss of intensity in the salicylic balsam and was unchanged in the other media.

Of the five, clarite appears to be the most useful mounting medium for the two stains. Liquid petrolatum, while showing nearly perfect preservation of Romanowsky staining, requires cementing of cover glasses, and has a considerable tendency to leak, creating an oily film not only on the adjacent part of the slide, but also over the margins of the cover glass for several millimeters.—R. D. LILLIE, Division of Pathology, National Institute of Health, Washington, D. C.

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

MICROSCOPE AND OTHER APPARATUS

DEROW, HARRY A. An inexpensive polarizing device for microscopes. *J. Lab. & Clin. Med.*, **26**, 694–5. 1941.

A search should always be made with a polarizing microscope for doubly refractile bodies in a urinary sediment in a case of massive albuminuria. An inexpensive type may be made as follows: Unscrew the outer lens mount of the ocular and apply Canada balsam to the rim on its under surface; attach an appropriate circle of polaroid film to serve as the analyzer; slip a wide circle of polaroid film into the slot under the condenser and leave it permanently to serve as a polarizer. Polaroid film may be purchased from The Polaroid Co., 285 Columbus Avenue, Boston, at $4.00 per 4 inch square. The polarizer and analyzer should be set at right angles by rotating the eye piece until there is a reddish-purplish-black field; then doubly refractile bodies appear as Maltese crosses. Such bodies occur in the nephrotic syndrome, associated with glomerulonephritis, amyloid disease, renal vein thrombosis, diabetes and hypertension. The microscope can be used for ordinary purposes by making the analyzer and polarizer parallel.—*John T. Myers.*

LOTZE, J. C., and YIENGST, M. J. An apparatus for measuring microscopic objects. *Science*, **93**, 45–6. 1941.

A device is described for obviating the shortcomings of micrometers and other scales when measuring objects less than 1 μ in diameter. Essentially, the apparatus consists of a microscope, the prism of a camera lucida, and a screen. The latter covers a lighted box, and is pierced with calibrated holes and slits that represent, as viewed thru the microscope, established lengths with which the size of microscopical objects can be compared and gaged.—*J. A. de Tomasi.*

MARTON, L. The Electron Microscope. *J. Bact.*, **41**, 397–413. 1941.

A description with diagrams of the instrument, a chart of its resolving power, photographs, and bibliography are included.— *Virgene W. Kavanagh.*

POHL, H. A. Convenient heat filter for tissue illuminator. *Science*, **92**, 612. 1940.

The quartz rod illuminator of Knisely used in the observation of tissues *in situ* transmits a considerable amount of heat from the illuminating bulb. The inconvenience of using a continuous stream of Ringer's solution as a cooling agent is eliminated by inserting a double thickness of inexpensive "heat filter" glass (Cenco 87305) at the break in the rod light conductor. The heat transmitted is cut to at least 1/7 its previous value, while the light intensity is reduced by only a small fraction.—*J. A. de Tomasi.*

RHEA, H. E. The new electron microscope. *Science*, **93**, 357–8. 1941.

The first electron microscope represented a major step toward fundamental developments in the field of microscopy; with it, particles of 50 Å in diameter could be clearly resolved. But that first apparatus was bulky and cumbersome, it required an installation free from vibrations and magnetic fields, and necessitated the services of an electronic engineer to operate it. The latest model is designed to fit into a small room and to be simple in operation. The microscope, including its power supply, is contained in one rack 7 ft. tall and weighs about 500 lb. A normal 110-volt line socket supplies the current.—*J. A. de Tomasi.*

SMITH, K. U. **A dry-ice freezing unit for cutting frozen sections.** *Science*, **92**, 364. 1940.

This unit is designed to obviate the shortcomings of commercially available attachments, and to meet such needs as sectioning of large specimens, and prolonged and uniform freezing. It consists briefly of a cylindrical cast-aluminum container of 8.4 cm. in diameter with a slightly raised and grooved center table 5 cm. in diameter. A hexagonal bakelite support is fitted underneath for clamping into the holder of the microtome. The tissue rests on the table, and powdered dry ice is packed around and under it within the container. This unit may be obtained from M. Hanford, Physics Department, University of Rochester, Rochester, N. Y.—*J. A. de Tomasi.*

MICROTECHNIC IN GENERAL

BUCHOLTZ, IRENE. **Cellophane coverslips and a method for mounting.** *Science*, **92**, 436. 1940.

Plain cellophane (No. 300) appears to be satisfactory as a substitute for cover glasses which are becoming increasingly scarce and expensive. This material has a refractive index of 1.53, comes in rolls 15/16 in. wide, and is 0.022 mm. thick. In favor of using it in microtechnic are its thinness, low cost, rapid drying, and the fact that it does not curl. It also allows taking photomicrographs, and the use of oil immersion except in the case of very high magnifications. One difficulty, however, lies with the problem of storing the cellophane. It tends to absorb moisture and the edges of the roll then wrinkle. Storage in a dessicator for long periods causes warping and cracking. Moisture-proof cellophane is less desirable than plain cellophane.

For mounting slides with glass or cellophane cover slips, proceed as follows: turn the slides face down on paper towels, pipet xylene between them, and cover with another towel. In 15–30 min. the preparations are ready for use.—*J. A. de Tomasi.*

GROAT, R. A. **Evaluation of isobutyl methacrylate polymer as a mounting medium.** *Science*, **92**, 268. 1940.

This is a discussion of the value of isobutyl methacrylate polymer as a mounting medium principally from the point of view of relative refractive indices. These values are as follows: unstained striated muscle, 1.537 ± 0.003; Canada balsam, 1.535; gum damar, 1.542; Clarite, 1.544; Clarite X, 1.567; isobutyl methacrylate polymer, 1.477. Since the refractive index of stained tissue is still higher than that of unstained tissue, the low value for the polymer appears still less compatible with the perquisites of the dispersion curve obtained by plotting refractive index against wave length. An ideal mounting medium cannot be realized because different tissue elements do not have the same dispersion curves; thus for practical purposes a medium should be used that has a refractive index for the D line of the spectrum as close as possible to that of most of the stained tissue elements. The use of the polymer for mounts with or without cover glass is not recommended for other reasons: it does not adhere to glass as well as other resins; certain stains fade rapidly in it; and it softens and decomposes at relatively low temperatures.—*J. A. de Tomasi.*

GROAT, R. A. **New paraffin resin infiltrating and imbedding media for microtechnique.** *Science*, **93**, 311–12. 1941.

A series of mixtures of paraffin (m.p. 56°–58° C.) is here reported to mix well with water-clear, LX–291, hydrocarbon resin produced by the Neville Co., Pittsburgh, Pa. Chief advantage is that by changing proportions in the mixtures, the resulting media differ appreciably in hardness at a given temperature, but not essentially in melting range. Hardness increases as the resin content rises. Concentrations suitable for practical application, containing 5, 10, 20, and 30% resin, show a melting range of several degrees, with a minimum around 55° C. An average oven temperature of 59° C. is satisfactory for all. The mixtures are prepared as follows: Blend the weighed portions of paraffin and resin at 170° C., and filter. Infiltrate in pure paraffin, or 5–10% mixtures; embed in a harder medium. For comparison, a 10% medium yields satisfactory ribbons in the range between 4 and 12 μ; a 50% medium permits only 1 μ ribbons.—*J. A. de Tomasi.*

McDOWELL, A. M., and VASSOS, G. A., JR. **Comparison of starch paste and albumin mixture as agents for the routine mounting of paraffin sections.** *Arch. Path.*, **29**, 432. 1940.

Albumin-glycerin mixture is made by taking equal parts of egg white and glycerin, filtering and adding a small crystal of thymol as preservative. Starch paste is made by adding 10 cc. of cold water to 1 g. of powdered starch and mixing thoroly. The mixture is then poured in 20 cc. of boiling water, 2 drops of dilute HCl are added and the suspension is boiled with constant sirring for 5 min. A small crystal of thymol is added when the paste is cool. After mounting the sections, the slides are submitted to various forms of drying; at 56°–58° C. for 48 hr. or at 38°–50° C. for 5 days is adequate for subsequent staining with silver but unnecessary for other technics; drying on a hot plate for 3 min. and in drying oven for 1 hr. at 56°–58° C. is adequate for Ziehl-Neelsen with ablumin but not with starch. (*From J. Royal Micro. Soc.*, **60**, 262, 1940.)

RANDALL, A., and MENZIES, A. W. C. **Histological sectioning of hard tissues by a new technique.** *Science*, **93**, 189–90. 1941.

The use of fluid methacrylates is suggested as a possible means of obtaining sections of hard and brittle materials like bone tissue. The resin is polymerized to a solid embedding medium *in situ*, and sections are ground from the specimen by petrographic technic. The procedure is as follows: Embed dry materials directly; or dehydrate (in alcohol, acetone, or dioxan), and clear in xylene. Impregnate in a test tube (sometimes at reduced pressure) in monomeric methyl methacrylate for 12 hr., changing it three times with 0.05% benzoyl peroxide added as a catalyst for polymerization. Heat a portion of the catalyzed monomer for 24 hr. at 40° C. Free the hardened contents of the test tube by breaking the glass, and proceed with the grinding. If serial sections are desired, cut first thin wafers and affix to "plexiglass" slides with the partial polymer; complete the polymerization for 6 hr. at 40° C. Sections obtained by this method are reported to stain well with safranin and methylene blue.—*J. A. de Tomasi.*

SUNTZEFF, V., and SMITH, IRENE. **The use of plastic as a substitute for coverglasses.** *Science*, **93**, 158–9. 1941.

These remarks are an extension to a note in Science, **92**, 17–8, 1940. It is stated that hematoxylin eosin stains mounted in Canada balsam will fade within 4–5 months when mounted under plastic covers.—*J. A. de Tomasi.*

DYES AND THEIR BIOLOGICAL USES

VARCO, RICHARD L., and VISSCHER, M. B. **Further studies in the elimination of certain dyes by gastric mucosa.** *Proc. Soc. Exp. Biol. & Med.*, **46**, 295–8. 1941.

The following list of dyes reported to be secreted in gastric juice has been reinvestigated: Congo red, orange G, mercurochrome, erythrosin, phloxine, rose bengal, lithium carmine, trypan red, eosin, and chrysoidin. None of them appeared in histamine-excited gastric juice from Pavlov or Heidenhain pouches in dogs. This work gives support to the earlier generalization that only dyes in which the chromogen may be in the cation appear in highly acid gastric secretions. —*M. S. Marshall.*

ANIMAL MICROTECHNIC

BAXTER, J. S. **The application of trichrome staining methods to embryological technique.** *J. Anat.*, **75**, 137–40. 1940.

This paper is a summary of seven years experience with trichrome staining methods in the preparation of several mammalian embryonic forms for teaching and research. A method for the preliminary treatment of embryos is given. Detailed procedures for two trichrome staining methods are outlined, together with the author's notes and comments. The author recommends for young embryos the use of an iron-hematoxylin nuclear stain followed by a simple cytoplasmic counterstain such as orange G in 1% solution. The embryos used in the above study were those in which epithelial and connective tissue differentiation had commenced.—*F. M. Clark.*

COULSTON, FREDERICK. **The use of diaphane for mounting Giemsa type preparations.** *Amer. J. Clin. Path.*, **26**, 869–73. 1941.

Smears and dehydrated sections often fade when mounted in balsam due to formation of acid by oxidation. Diaphane does not become acid. It is unconditionally recommended instead of balsam or cedar oil for mounting preparations with any Romanowsky type stain.—*John T. Myers.*

GORDON, H. K., and CHAMBERS, R. **The particle size of acid dyes and their diffusibility into living cells.** *J. Cellular and Comp. Phys.*, **17**, 97. 1941.

A study was made of the relation of the particle size of a number of water-soluble acid dyes to their penetration into three types of living cells. The tissues used were muscle fibers of the gastrocnemius, ciliated epithelial cells of the oral mucosa and sheets of bladder epithelium of the frog *Rana fusca*. Dyes with a particle radius of 5.5 ± 0.4 to 6.4 ± 0.5 Å (when diffusing in Ringer's solution at pH 7.4 to pH 6.8) or less penetrated the ciliated epithelial cells; the upper limit of size for the muscle fibers or the bladder epithelium was 6.5 ± 0.4 to 7.3 ± 0.9 Å. No definite relation was observed between lipid solubility and the penetrability of the dyes. No difference in penetration of the dyes was observed for cells under a nitrogen or an aerobic atmosphere.—*L. Farber.*

KATZ, ALBERT. **A contrast staining method for hemosiderin pigment in heart-failure cells.** *J. Lab. & Clin. Med.*, **26**, 700. 1941.

There has been no satisfactory method for differentiating heart failure cells, in sputum or urine, from the surrounding leucocytes. This can be done by the following technic:

Make smear of sputum as usual, air-dry, fix with heat, apply 10% K_4FeCN_6 and an equal quantity of 10% HCl. Let stand 15 min., wash with water and dry. Apply Wright's stain and add buffered water for 5 min. Wash with water, dry and examine under oil. The hemosiderin takes a bright green stain.—*John T. Myers.*

LENDRUM, A. C. **The preparation of tissues for paraffin embedding.** *J. Path. & Bact.*, **52**, 138–42. 1941.

The author finds Stiles' butanol dehydration series (Stain Techn., **9**, 97, 1934) the most satisfactory among a wide variety of methods for preparing easily sectioned paraffin blocks. Tissue can be left for days in the dilutions without harm, and a prolonged stay afterwards in the paraffin oven does no harm. Better results with dense tough tissues can be obtained by following butanol dehydration with pyroxylin infiltration as follows: equal parts of butanol and methyl benzoate, one day; methyl benzoate, one day; then 7–14 days in methyl benzoate, containing 2% pyroxylin and 1.5% tricresyl phosphate; two changes of chloroform, over a period of 5 hr., to precipitate the pyroxylin; infiltration with paraffin for 6–48 hr. or longer.

Low viscosity nitrocellulose can be used if combined with sufficient plasticiser, by the following technic: Pass tissues from butanol, thru two graded mixtures, to pure cellosolve, then to a 15% solution of collodion cotton in cellosolve containing 14% tricresyl phosphate, and leave for 7–30 days. Then treat with chloroform and imbed in paraffin as usual. Without plasticiser the collodion cotton is much too hard.—*S. H. Hutner.*

MULLEN, JOHN P., and McCARTER, JOHN C. **A mordant preparing formaldehyde-fixed neuraxis tissue for phosphotungstic acid hematoxylin staining.** *Amer. J. Path.*, **17**, 289–91. 1941.

The authors propose a method of mordanting formalin-fixed tissue, even after storage in formalin for several years, which yields results equivalent to those obtained on tissue fixed in Zenker's fluid. The procedure is as follows: Wash the fixed tissue thoroly and prepare it for paraffin sections in the usual manner. After cutting and affixing the sections to the slide, remove paraffin and pass the slides thru graded alcohol to water. Mordant for 2–48 hr. in a 5% solution of $CrCl_3 \cdot H_2O$, (green crystals; obtainable from the General Chemical Co., New York, N. Y.) in distilled water to which 5% glacial acetic acid is added. (This mordant is stable and will keep for weeks, altho the color changes.) Rinse, after mordanting, in distilled water, treat with 0.25% aqueous $KMnO_4$ for 10–15 min., rinse again,

and bleach in 5% aqueous oxalic acid until the sections have lost the brown color; rinse and stain with Mallory's phosphotungstic acid hematoxylin (either the newer or older formula) for 6–12 hr. Decolorize in 95% alcohol, 2 or 3 changes; dehydrate; clear and mount. This method is suited for routine use on brain and spinal cord.—*H. A. Davenport.*

OTTO, REDGINAL HEWITT, OTTO, C. F., and STRAHAN, DOROTHY E. **A simplified zinc sulfate levitation method of fecal examination for protozoon cysts and hook worm eggs.** *Amer. J. Hyg.*, **33**, 32–7. 1941.

The technic is that of the Willis brine levitation method (Med. J. Austr., 1921, 375–6) except that 1.18 sp. gr. $ZnSO_4$ is substituted for saturated NaCl solution. A portion of feces is placed in a 1 oz. metal container 3.8 cm. in diameter and 2.8 cm. deep. It is completely filled with the $ZnSO_4$ solution, covered with a glass slide and let stand 1 hr. The slide is removed, inverted and examined. The method cannot be used for the vegetative stage of amoebae.—*John T. Myers.*

SALTER, W. J. **A haemocytometer method for reticulocyte and platelet counts.** *J. Path. & Bact.*, **52**, 148–50. 1941.

Reticulocytes and platelets can be counted simultaneously and rapidly by the following accurate method: Prepare the diluting fluid by mixing two parts of an aqueous solution of brilliant cresyl blue (1:300) with three parts of a 1:1,400 aqueous solution of KCN, the final solution being centrifuged at high speed for 5 min. (The two solutions must be kept separate and mixed immediately before use.) Take blood to the mark 0.5 in a white cell pipette, dilute to mark 11 with the diluting fluid, and shake well. Place a drop in the counting chamber and count after 5–10 min.

Platelets appear as ovoid blue or lilac-colored bodies. Reticulocytes are recognized as usual. A leucocyte count can be made at the same time.—*S. H. Hutner.*

SMITH, KATHERINE EDSALL. **The measurements of red blood cell diameter by the diffractometer.** *J. Lab. & Clin. Med.*, **26**, 696–699. 1941.

The Spencer Lens Co. manufactures a diffractometer which may be attached to any monocular microscope. A small black cylinder with a pinhole in the bottom is attached in place of the objective. A second cylinder with a pinhole in the top and an adjustable device on the side for measuring the halo replaces the ocular. A stained or unstained slide may be inserted into a slot in the top cylinder with the smear down. The smear should be only one cell thick, and should be observed with maximum light. A central spot of light should appear, surrounded by a yellow halo which shades into a red periphery. The two parallel black bands should be adjusted on the outside so that they just touch the outer rim of the red halo. The reading is taken from the sliding scale and the mean cell diameter determined from a conversion table. This method is useful in determining macro- or microcytosis.—*John T. Myers.*

TARAO, S. **Microchemical studies on the Golgi apparatus using protease Nile blue sulfate technique. II. Golgi apparatus of pancreatic acinar cells in the mouse in fixed and living conditions.** *Cytologia*, **11**, 261–81. 1940.

Anilin dyes are not ordinarily considered useful for staining the Golgi apparatus, but Nile blue sulfate was used to demonstrate it in a series of papers by Tarao of which this is the third. The protease Nile blue sulfate technic is applied as follows: Fix small pieces of the pancreas of a mouse not fed for 10 hr. (anaesthetized with chloroform) in 20% neutral formalin (8% formaldehyde) for 24 hours or more. Make 20 μ sections on a freezing microtome. Digest for about 16 hr. at 37° C. in a solution of 0.3 g. trypsin and 0.3 g. anhydrous Na_2CO_3 in 100 cc. distilled water, to remove much of the protein from the ascinar cells and the masked proteins of the Golgi apparatus. Wash thoroly in distilled water and stain over night in 0.01% aqueous Nile blue sulfate (Grübler's). Differentiate in 1% aqueous acetic acid until the sections are faded to a cobalt blue. Wash thoroly in distilled water. Mount in a saturated aqueous solution of dextrose and seal with vaseline-paraffin in equal parts. The Golgi substance stains pink-blue; other fatty substances stain red or blue according to their degree of saturation; the cytoplasm is unstained. The Golgi apparatus is the same shape and size as it is after metallic impregnations. Pepsin solutions may be used to digest the

hepatic cells of mouse and newt but do not digest the pancreatic cells. Other fat stains may be substituted for the Nile blue sulfate; Smith-Dietrich's hematoxylin, Ciaccio's Sudan III, and Fischler's copper-acetate-hematoxylin give rather good results.

Intra-vital staining of the Golgi apparatus is secured by injecting 0.6–0.7 cc. of 0.2% Nile blue sulfate in normal saline intraperitoneally in the mouse. (A slightly larger dose would be fatal.) In about 30 min. the Golgi apparatus develops a faint blue tinge which lasts not longer than 20 min.; the bits of pancreas must be removed, mounted, flattened, and examined within this period. Polarized light is useful for viewing these slide preparations of bits of the pancreas. Thru a Zettnow's green filter, which is made up of $CuSO_4$ and CrO_3 in distilled water, the blue color of the Nile blue sulfate appears black. Other small granules (Ries' Lyrochondria) are stained a dark blue in the same preparations.— *Virgene W. Kavanagh.*

WETMORE, PSYCHE W. **A direct method of determining the erythrocyte, leucocyte and thrombocyte count of fowl blood.** *Science*, **92**, 386. 1940.

This technic is a modification of the Blain method, and affords rapid and reliable enumeration of the cellular elements of avian blood in the counting chamber. The procedure is as follows: Prepare a staining stock solution by mixing 1 cc. 1% aqueous brilliant cresyl blue with 0.25 cc. saturated aqueous pyronin (6.7%); dilute 0.2 cc. of this in 25 cc. normal saline, and filter thru neutral paper. Draw blood from the wing vein up to the 1 mark in the red cell counting pipette. Fill it half way with the dye-saline solution, rotate 5 sec., and fill to the 101 mark with 12% formalin in Locke's solution, as a preservative. Shake for 30 sec.; for better differentiation, allow it to stand 15 min. or longer. Use thin covers, and examine under a 4 mm. objective. The dye solution keeps for about a week, while the stock solution is less stable; the formalin solution must be made fresh each day.—*J. A. de Tomasi.*

WU CHOA-FA. **Laboratory technique notes. III. The use of alum-hematoxylin for staining in bulk.** *Peking Nat. History Bull.*, **15**, 135–7. 1940.

Most methods of staining large blocks of tissue have used carmine or have required an extensive treatment of the slides after sectioning. The following method apparently gives well-stained sections without any treatment which might wash them off the slides: Place blocks of animal tissue, that have been fixed in Bouin's fluid, washed, and decolorized as usual, in about 20 volumes of hematoxylin solution (8 cc. of Ehrlich's stock solution of hematoxylin, 30 cc. of 50% alcohol, and 2 cc. of glacial acetic acid) for 2–5 days, 2 days for a piece 3 mm. on an edge, 5 days for a centimeter cube; stir occasionally; rinse the block in water; to remove the excess stain, soak it in 3 or more changes of weak acetic alcohol (5 cc. of 10% acetic acid in 100 cc. of 30% alcohol) until the last change remains almost colorless for ½ hr.; wash in running tap water over night; and dehydrate in a series of alcohols to 95%. If a counterstain is desired, place the block in a 0.2% solution of alcohol-soluble eosin in 95% alcohol, then in a 0.2% solution in absolute alcohol, 12 to 24 hr. in each. After the blocks have been stained, they may be cleared and embedded as usual altho the following method is suggested: Pour a layer of chloroform twice as deep as the thickness of the block into a vessel; on top of this pour a similar layer of methyl salicylate; and on this pour a layer of absolute alcohol; put the block into the alcohol layer and let it remain in the vessel until it has sunk into the chloroform; put it into paraffin-chloroform mixtures, then into paraffin as usual for embedding. The methyl salicylate layer may be omitted for small embryos and delicate tissues. After the blocks are embedded, section and mount as usual; then remove the paraffin with xylol, run the slides to absolute alcohol for 1 min. or more to differentiate the eosin; run them back thru xylol and mount in balsam.— *Virgene W. Kavanagh.*

ZAHL, P. A., and COOPER, F. S. **Localization of lithium in tumor tissue as a basis for slow neutron therapy.** *Science*, **93**, 64–5. 1941.

It has been repeatedly observed that certain acid dyes, when introduced into the blood stream, accumulate preferably in cancer tumor tissue. Since most of these dyes are sodium salts of the azo-sulfonic complex, the effect of substituting lithium for sodium in the dye-salt molecule is investigated. The reason for doing

this is that slow neutrons projected thru body tissue cause no damage when they encounter a zone perfused with such elements as boron or lithium. In this case nuclear capture reactions release protons of high energy and the result is local destruction of tissue, suggesting application of a neutron-boron or neutron-lithium technic for localized treatment of tumors. Lithium salts of pontamine sky blue 6B, trypan blue, and carminic acid (duPont and National Aniline Co.) were prepared and injected intravenously in mice with spontaneous and implanted mammary tumors. Spectroscopical analysis of tissues removed later proves that a localization factor of approximately 2 is attained, with a maximum concentration of 0.01–0.03% lithium in the tumor mass. The addition of lithium-ions results in a net increase in absorbed energy corresponding to a maximum gain of about 43% in the radiation dosage of the tumor over that of other tissues in the same mouse.—*J. A. de Tomasi.*

MICROÖRGANISMS

BESTA, B. L'uso del microscopio a fluorescenza nella ricerca del bacillo di Koch. *Ann. d. Ist. Carlo Forlanini*, **3**, 904. 1939.

The method of Keller was used, with a 2:1000 solution of auramin. In 200 examinations of material already known to be positive by the Ziehl-Neelsen method, the fluorescence method gave the same positive results, but required less time for the examination of the slides because a larger microscopical field could be covered by the use of small power objectives, i. e. 10× or 20×. In 241 examinations of previously negative material, 7% were positive by the Ziehl-Neelsen method, 18.7% by the fluorescence method, and 34% by culture on Petragnani's medium or by guinea pig inoculation. This method is time-saving, more accurate and economical, because in less than 50% of cases biological proof is needed. Occasional doubtful results may be had with the fluorescence method, but this is also true of the usual staining methods.—*S. Lojacono.* (*From Abst. sect. of Amer. Rev. Tuberc.*, **43**, *No.* 4, 1941.)

DIDION, H. Über den fluorescenzmikroskopischen Nachweis von Tuberkelbakterien. *Klin. Woch.*, **18**, 1315. 1939.

Fluorescence microscopy has been employed as an alternative method of identifying tubercle bacilli on slides. The preparation is stained with auramin, a yellow anilin dye; when viewed under the microscope, the organisms are colored a yellowish green. In order to determine the accuracy of this method, 702 specimens, including sputum, pus, urine, feces and other body fluids of suspected tuberculous nature, were examined for tubercle bacilli by four methods: animal inoculation, culture (according to the method of Löwenstein, Lockemann and Hohn), Ziehl-Neelsen stain, and auramin stain according to the method of Hagemann. Altogether there were 157 positive specimens: 129 by the Ziehl-Neelsen method; 137 by the auramin method; 12 were discovered by cultural methods; and 8 only by animal inoculation. Of the 157 positive specimens, the following figures show the percentage accuracy of the various methods: animal inoculation, 100; culture, 92; auramin, 87.2; Ziehl-Neelsen, 82.2. Of the staining methods the auramin fluorescence method is not only somewhat more accurate but is also definitely time-saving, particularly in searching for tubercle bacilli in tissue preparations.—*H. R. Nayer.* (*From Abst. sect. of Amer. Rev. Tuberc.*, **43**, *No.* 4, 1941.)

KNAYSI, G. Observations on the cell division of some yeasts and bacteria. *J. Bact.*, **41**, 141–53. 1941.

To stain the cell walls and slime-layers, which are rarely seen in ordinary preparations, the following procedure is recommended: Smear a young culture (only a few hours old) and fix it over the flame; mordant it for 10 min. with a mixture of 70 ml. saturated aqueous $AlK(SO_4)_2$ and 30 ml. of 20% aqueous tannic acid; stain with Ziehl-Neelsen's carbol fuchsin under a cover glass. The cytoplasm is dark red; the cytoplasmic membrane, still darker; the cell wall, blue; and the slime-layer, bright red. If the preparation is sealed with vaspar, a mixture of 50% paraffin and 50% vaseline, it will keep for a considerable time. A fresh mordant mixture should be prepared every two weeks.—*Virgene W. Kavanagh.*

LIPP, HANS. **Ersparnisse bei der Gonokokken- und Spirochätenfärbung.** *Münch. Med. Woch.*, **87**, 888. 1940.

Staining procedures for gonococci and spirochaetes are given—the former an adaptation of the Thimsche technic.

The procedure for gonococci is as follows: Prepare a thin smear of the secretion and fix by passing it three times thru a flame; stain 30 sec. in a solution made up of equal parts of 1% aqueous methylene blue and 1% aqueous gentian violet solutions; wash and dry. The gonococci stain dark violet, the nuclei of leucocytes appear dark blue, the cytoplasm of leucocytes stains pale reddish-violet and the nuclei of epithelial cells deep blue.

Four staining procedures are described for spirochaetes, particularly for *Treponema pallidum*. These are as follows:

1. Fix smear in methyl alcohol; treat with a few drops of 1% KOH, followed immediately by a few drops of a dilute fuchsin solution (a 1:20 dilution of a saturated fuchsin solution in 95% alcohol) which becomes cloudy after 1 min. and is decolorized after 3 min.; wash in water and dry. (The red cells of *Treponema pallidum* are easily distinguished from the pale red background.)

2. Fix smear by drying in air, stain 3 min. in a 5% aqueous $KMnO_4$ solution; wash with water; counterstain 2 min. with a 1:10 carbol fuchsin solution. (The *Treponema refringens* and mouth spirochaetes stain a deep red brown, *T. pallidum* stains somewhat lighter but still distinguishable.)

3. Prepare a hanging drop of the serum obtained by the irritation of an infected area; add 1 or 2 loopfuls of Loeffler's methylene blue; mix and add 1 loopful of N/10 NaOH. (*Treponema pallidum* appears sky blue and is to be found particularly at the edge of the drop.)

4. Air-dry the smear; treat with 3% aqueous Victoria blue IV R (Grübler) 3 min.; wash carefully and dry in the air. The characteristic morphology of *Treponema pallidum* is clearly distinguishable from that of any other spirochaetes which may be present. A more intensely staining solution may be prepared as follows: Victoria blue IV R, 3 parts, pyronin 0.9 parts, methyl green 0.1 part, absolute alcohol 9 parts, glycerol 10 parts and distilled water to 100 parts. This solution stains *Treponema pallidum* a deep blue-black color in 3 min. —*L. Farber.*

MALLMANN, W. L., and DARBY, C. W. **Uses of lauryl sulfate tryptose broth for the detection of coliform organisms.** *Amer. J. Pub. Health*, **31**, 127–34. 1941.

The addition of sodium lauryl sulfate, a surface tension depressant, to lactose broth gives a medium selective for the coliform group. The base medium consists of 2% Bacto tryptose; 0.5% NaCl, 0.4% K_2HPO_4, 0.15% KH_2PO_4, 0.5% lactose. To this is added sodium lauryl sulfate, as Duponol W. A. Paste or Nacconol N. R. F. S., to give a concentration of 1 part in 10,000. A comparison was made by testing three media in parallel, namely, standard lactose broth, lactose tryptose broth, and lauryl sulfate tryptose lactose broth. Most of the studies were on raw waters. Confirmation was carried out in either brilliant green bile broth or E. M. B. agar or in both. The results showed that, with unpolluted tap waters, standard lactose broth gave many more positive presumptive tests which failed to confirm than did lauryl sulfate broth or tryptose broth. With polluted raw waters, there was little difference between lactose broth and lauryl sulfate broth as regards number of positive gas tubes and tubes confirmed, altho the latter allowed the growth of more gas producers than did the former. The confirmatory media often acted as suppressing agents to coliform organisms, giving a lower coliform index than would be the case with more suitable confirmatory media. Tryptose broth was a better confirmatory medium than brilliant green broth. When gas was produced in lauryl sulfate broth, confirmation was always obtained.—*M. W. Jennison.*

MAYFIELD, CATHERINE R., and GOBER, MAUD. **Comparative efficiency of plating media for the isolation of Shigella dysenteriae.** *Amer. J. Pub. Health*, **31**, 363–8. 1941.

This paper reports the comparative efficiency of plain Endo, lithium chloride Endo, desoxycholate-citrate, bismuth sulfite, and Shigella Salmonella culture media for the isolation of *Shigella dysenteriae* from routine feces specimens re-

ceived at the laboratories of the Mississippi State Board of Health. The Shigella Salmonella medium is a recent product of the Difco Laboratories. Specimens were inoculated on the plating medium used for isolation the day they were received, and repeated platings made on the second and third days. After 18 hours incubation suspicious colonies were fished to triple sugar tubes, and colonies positive on this medium were inoculated into various carbohydrate media. Motility tests, as well as indole determinations and macroscopic slide agglutination tests, were made. Of 435 positive cultures isolated on one or more of the media, there were 95% positive on desoxycholate-citrate, 48% on plain Endo, 20% on lithium chloride Endo, and 2.3% on bismuth sulfite; of these, 175 were positive only on desoxycholate-citrate, compared with 14 from plain Endo, 4 from lithium chloride Endo, and 2 from bismuth sulfite agars.—*M. W. Jennison.*

MIYAHARA, H. **The quick staining method of the malaria parasite by means of Giemsa's stain.** *Acta Jap. Med. trop.*, **1**, 49–55.

The dried unfixed film is hemolysed in a beaker of water, the film side of the slide being uppermost. The solvent of the stain, 0.003–0.005% K_2CO_3 or Na_2CO_3 solution, is heated to about 60° C. One drop of the Giemsa stain is added to each cc. of the heated solvent; 3 cc. of the warm solution is poured on the film and left for 5–10 min.; the film is then washed and dried. Schüffner's dots are well stained but appear finer than after alcohol fixation. The parasitized cells and the parasites are well stained. No stain granules are deposited on the film. (*From J. Roy. Micr. Soc.*, **60**, 262, 1940.)

MONK, C. R. **Marine harpacticoid Copepods from California.** *Trans. Amer. Micr. Soc.*, **60**, 75–99. 1941.

The harpacticoids, usually killed by collecting them in fresh water or in 2% formalin, were stained with fast green (no other information given) in 70% alcohol for 1 hr. before dissection. The addition of a few drops of Bouin's fluid to the stain aided in distinguishing fine setae. They were dissected in glycerin.

Permanent mounts of the appendages were made in a syrup-pectin mixture (white Karo syrup, 5 cc.; Certo, 5 cc.; water, 3 to 5 cc.), dried over heat, covered with euparal and bits of cover glass.— *Virgene W. Kavanagh.*

RICHARDS, O. W. **The staining of acid fast tubercle bacteria.** *Science*, **93**, 190. 1941.

Of the many compounds isolated from tubercle bacteria, mycolic acid alone was found to be acid-fast; but the degree of fastness varies greatly according to the dye. The carbol fuchsin of the Ziehl-Neelsen method stains it with difficulty, and is easily washed out in acid alcohol; carbol auramin, on the other hand, stains the acid an intense fast yellow. While mycolic acid is weakly fluorescent to ultra-violet irradiation, auramin causes it to show bright yellow fluorescence. The suggestion is made that mycolic acid may be the source of acid-fastness, and may be responsible for higher counts in the flourescence technic.—*J. A. de Tomasi.*

RICHEY, DALE. **Relative value of 2% and 5% brilliant green bile confirmatory media.** *J. Amer. Waterworks Assoc.*, **33**, 649–58. 1941.

It was found that this medium was specific for the coliform group of organisms, with either 2% or 5% brilliant green. The latter was superior to the former in (1) the number and proportion of coliform organisms isolated, (2) giving the smallest total cumulative error, and (3) inhibiting spore-forming organisms.—*Merritt N. Pope.*

SCHLEIFF, P. **Zur Pilzfärbung, insbesondere der Erreger der Epidermophytien der Hände and Füsse.** *Münch. Med. Woch.*, **87**, 785. 1940.

A method is described for the preparation and staining of samples for the detection of epidermophytic infections.

Preparation of material. Scraping from around the margin of non-purulent areas of infected skin or the tops of vesicles are transferred to a slide. They are treated 3 to 10 min. (depending on the size and thickness of the specimens) with Carnoy's solution (glacial acetic acid 10 ml., chloroform 30 ml., absolute alcohol to make 100 ml.). This treatment serves to swell the horny substance of the skin,

to remove any fatty material and to dehydrate the sample. The Carnoy solution is poured off carefully in order to carry away any floating debris and leave the fungus adhering to the slide. Dry carefully over a flame.

Staining Procedure. Treat with a 0.5% aqueous Azure I solution 2–3 min.; wash carefully with distilled water, adding the water drop by drop to avoid washing away the sample. Dry over a flame and mount in Canada balsam.

The fungal filaments stain dark blue and are easily distinguished from the light blue background.—*L. Farber.*

SCHNEIDER, P. P. **Fluoreszenzmikroskopie und Tuberkelbazillennachweis im Tuberkulosenkrankenhaus.** *Zts. Tuberk.*, **84**, 319. 1940.

The advantages of the fluorescent microscope in demonstration of tubercle bacilli have been previously reported. There is considerable saving of time and less fatigue. In a series of 1100 examinations there were 22.7% more positive results with the fluorescent microscopy than with the Ziehl-Neelsen method. Negative in relation to positive results were: for sputum, 3:4 with Ziehl-Neelsen, and 3:5.5 with the fluorescent microscopy; for antiformin concentrate, 3:0.9 and 3:1.4; for gastric juice, 9:1 and 8:1. The use of the method in the tuberculosis hospital is highly recommended, but it cannot replace culture and animal expts.—*G. C. Leiner.* (*From Abst. sect. of Amer. Rev. Tuberc.*, **43**, *No.* 4, 1941.)

STANLEY, I. N. **Development of the basidium of Eocronartium muscicola.** *Trans. Amer. Micr. Soc.*, **59**, 407–13. 1940.

Dried basidia have been stained well with phloxine by the following technic: Soak sporophores for a few hours in water; tease out minute particles of the hymenium on a slide; add 3% KOH; add 1% phloxine (no other specifications); cover; remove excess phloxine by pulling KOH under the cover glass with filter paper. By this method, basidia are bright rose; sterile hyphae, light pink. Lactic acid proves useful in swelling the dried material.— *Virgene W. Kavanagh.*

STARR, M. P. **Spirit blue agar: a medium for the detection of lipolytic microorganisms.** *Science*, **93**, 333–4. 1941.

This is a medium claimed to be highly differential and at the same time to be capable of allowing growth of the more delicate microorganisms as well. The preparation of the medium is as follows: Dissolve 30 g. agar, 10 g. tryptone, and 5 g. yeast extract in approximately 900 ml. distilled water by autoclaving; to this add 25 ml. of a 20% cottonseed oil emulsion (10 g. powdered gum arabic ground thoroly in 100 ml. Wesson oil and 400 ml. warm distilled water), and 50 ml. of 0.3% freshly filtered alcoholic spirit blue (National Aniline); bring up to 1000 ml. with distilled water, and autoclave 15 min. at 15 lb. (121° C.). The medium must be stored in a refrigerator till used. Plates made from it appear pale lavender; lipolysis is indicated by a permanent deep blue beneath and around the colony.—*J. A. de Tomasi.*

TYAGARAJA, S. **A simple modification of Wilson and Blair's medium for the isolation of typhoid and paratyphoid bacilli.** *J. Hygiene*, **40**, 414–22. 1940.

To heart infusion agar at 60° C. is added, per liter, 0.1 cc. of 1% brilliant green, 4 cc. of 12% fresh bismuth ammonium citrate and 4 cc. of 40% Na_2SO_3, shaking after each ingredient is added. Glucose, phosphate and $FeSO_4$ are eliminated. Good keeping qualities, inhibition of *Aerobacter aerogenes*, and differentiation are claimed.—*M. S. Marshall.*

WENRICH, D. H. **The morphology of some protozoan parasites in relation to microtechnique.** *J. Parasitology*, **27**, 1–28. 1941.

The author emphasizes the importance of properly prepared slides as an aid in the diagnosis of intestinal protozoa, and discusses the value of modifications of the usual technics.

Fixation for 1 min. gives results as good as or better than longer times for the fixing agents commonly employed for intestinal protozoa. Schaudinn's fluid and other fixatives can be greatly diluted without destroying their effectiveness as fixing agents.

Different races of the same species may have different appearances with the same technic. Different individuals on the same slide may show different reactions to the same technic. On the other hand, different fixing solutions and different stains may produce decidedly different appearances for the same species, as, for example, *Trichomonas muris*. Different species show both chemical and morphological differences in the composition of their nuclei, a point particularly well shown when the Feulgen reaction is applied to the intestinal amoebae of man and the results compared with those obtained with the more common stains.—*Elbert C. Cole.*

HISTOCHEMISTRY

SEKI, M., and KOHASHI, Y. **Bestimmung des isoelektrischen Punktes der Gewebselemente auf färberischem Wege. I. Ergebnisse der pHi-Bestimmung in Anwendung verschiedener Puffergemische.** *Folia Anat. Jap.*, **19**, 47–52. 1940.

The authors have investigated the variable results obtained by others with the Pischinger's colorimetric method. They arrive at the conclusion that the composition and concentration of the buffer mixtures play an important role in the colorimetric isoelectric point determination (denoted by the authors as pHi) of tissue elements.

Clark's phthalate buffers, mixed with an equal amount of dye solution, proved the best, because their negativating anion action upon the tissue element charge was the lowest within the range pH 2.5–6.0. McIlvaine's Na_2HPO_4-citric acid mixture (diluted 5 times) served almost equally well.

Pieces of rabbit kidney, duodenum, and liver were used as test objects because of their loose texture and lipoid scarcity. Blocks were fixed 24 hr. in 95% alcohol or 10% formalin, passed thru 80%, 95%, and absolute alcohol into Dekalin-methylbenzoate-paraffin. Sections cut at 10 μ were stained for 30 min. in a mixture of dye solution and buffer in equal parts. The following Grübler dyes were used in the concentrations indicated: 0.01% ponceau P. R. or bordeaux red (both acid); 0.005% toluidine blue or safranin (both basic). The sections were washed for 2–3 sec. in buffer of the same pH and dried by blotting in such a way as to avoid wrinkling or too complete air-drying of sections; they were dehydrated with xylol and mounted in balsam.

The pH-range of rapid change in color intensity is the isoelectric zone of the tissue element investigated; its mean value is regarded as the isoelectric point (pHi). The intensity of color was estimated numerically with a color scale as follows: To a series of four test tubes were added respectively 4 ml. dye in concentrations of $0.01\% \times 0.8$, $0.01\% \times 0.8^2$, $0.01\% \times 0.8^3$, etc. The basic dyes were diluted with an equal amount of alcohol. A color scale was then sketched for comparison with the depth of the tone of the stained tissue. The article should serve as a working basis for those interested in isoelectric point determinations. Tables showing results with the various buffer mixtures mentioned are given.—*J. M. Thuringer.*

SEKI, M., and KOHASHI, Y. **Bestimmung des isoelektrischen Punktes der Gewebselemente auf färberischem Wege. II. Versuche an dicht strukturierten und lipoidreichen Gebilden.** *Folia Anat. Jap.*, **19**, 53–7. 1940.

The colorimetric method of pHi determination proved unsuitable for certain bacteria, and for the lecithin of the myelin sheath of nerves. The negative results are attributed to the dense texture of the structures and the presence of a large amount of lipoid substances.—*J. M. Thuringer.*

SEKI, M., and CHIN, K. **Bestimmung des isoelektrischen Punktes der Gewebselemente auf färberischem Wege. III. An Embryo von Huhn.** *Folia Anat. Jap.*, **19**, 59–64. 1940.

The isoelectric point was determined in cells of chick embryos of 3, 4, 5, 6, 7, 8, 9, 10, 13, 17, and 21 days incubation. The isoelectric point of the cells moved definitely toward the less acid side, a little more pronounced in the cytoplasm than in the nucleus. The rate of the changes was more rapid in the earlier stages than in the latter. The pH of the various cells differed very slightly in the earlier stages.—*J. M. Thuringer.*

SEKI, M., and KOHASHI, Y. **Bestimmung des isoelektrischen Punktes der Gewebselemente auf färberischem Wege. IV. An Kaninchenembryo.** *Folia Anat. Jap.*, **19**, 65–8. 1940.

In the germ layer of the rabbit embryo the isoelectric pH-value of the cells was mesoderm > entoderm > ectoderm. The epithelial cells of the yolk sac and the allantois as well as the syncytium of the chorionic villi have a relatively high pHi value. The isoelectric point of the ground substance of hyaline cartilage gradually moves toward the more acid side in contrast to most tissue constituents. The same may be said to a lesser extent in application to the gray and white matter of the spinal cord.—*J. M. Thuringer.*

SEKI, M., and CHIN, K. **Bestimmung des isoelektrischen Punktes der Gewebselemente auf färberischem Wege. V. An Epithelien des oberen Luftweges und oberen Verdauungskanals.** *Folia Anat. Jap.*, **19**, 113–6. 1940.

The local variations in the isoelectric point of the epithelia of the upper respiratory passages and upper alimentary canal were investigated. Epithelia having great resistance toward mechanical injury such as the stratified squamous epithelium of the gums, cheek, and lip had higher isoelectric points. This was attributed to the dense texture of the structures caused by syneresis of the cell colloids; correspondingly a lower isoelectric point was shown by the stratified squamous epithelium with lesser textural density over the regions of the lymph nodules.

The isoelectric point of formalin fixed and preserved material diminishes somewhat with time, the change being greater in the cytoplasm than in the nucleus.—*J. M. Thuringer.*

SEKI, M., and MUKOHATA, J. **Bestimmung des isoelektrischen Punktes der Gewebselemente auf färberischem Wege. VI. An Epithelien der männlichen Urogenitalorgane.** *Folia Anat. Jap.*, **19**, 117–20. 1940.

Formalin-fixed human material gave the following isoelectric points:

1. Epithelium of proximal convoluted tubules of kidney (characterized by abundance of basic substances) had a high isoelectric point (4.8). 2. The superficial cells of the fossa navicularis of the male urethra and the trigon of the bladder had the highest isoelectric point (4.85) of all. 3. The cells of Sertoli have a higher isoelectric point (4.70) than the spermatogonia and spermatocytes (4.60 and 4.65, respectively).—*J. M. Thuringer.*